

## ACCEPTED ARTICLE

# Genome-wide association mapping revealed SNP alleles associated with resistance to cereal cyst nematode (*Heterodera filipjevi*) in wheat

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## Abstract

Resistance traits are economically important in crops in terms of accessibility to promising resistant germplasms. This study was conducted to evaluate SNP marker-trait association for cereal cyst nematode (CCN), *Heterodera filipjevi* in a large number of natural bread wheat populations. Phenotypic data analysed using GLM (Generalized Linear Model) indicated significant differences among the landrace accessions for resistance to *H. filipjevi*. The genotyping was performed by 152K SNP chip on 188 accessions. After filtering, 10,471 polymorphic SNPs were employed for Genome Wide Association Study (GWAS). Population structure among the wheat genotypes were investigated using 840 well distinct SNP markers. Two sub-populations were revealed by structure software, and eleven markers were found to be significantly ( $p$ -value  $< 0.001$ ) associated with resistance to *H. filipjevi* on chromosomes 2A, 3B, 4A, 4B, 5A, 5B, 5D, and 6B. The linkage disequilibrium analysis for all significantly associated SNPs showed that markers on chromosomes 4A and 4B were in high intra-chromosomal linkage disequilibrium, and consequently, eight markers were recommended as strongly associated with resistance to *H. filipjevi*. The present study demonstrated valuable sources of resistance in the studied wheat genotypes against a widespread and important species of CCNs. The associated markers could be used in molecular breeding programs of bread wheat.

**Keywords:** Association mapping, *Heterodera filipjevi*, GWAS, SNP, Wheat.

## INTRODUCTION

Cereal Cyst Nematodes, CCNs (*Heterodera* spp.) are one of the most important causal agents of yield losses on wheat annually, hence its global importance is known in most wheat-growing

41 areas (Smiley *et al.*, 2017; Toumi *et al.*, 2018). The genus *Heterodera* is divided into nine  
42 groups based on morphological and molecular characteristics (Handoo and Subbotin, 2018), in  
43 which *H. filipjevi* is one of the most important species belonging to Avenae group. Host plants  
44 of *H. filipjevi* include wheat, rye, barley, corn, and many grasses (Smiley *et al.*, 2017). Yield  
45 losses caused by *H. filipjevi* in three winter wheat cultivars in Iran were estimated to be 20.4  
46 to 24.8% (Karimipour Fard *et al.*, 2018). Wheat is one of the world's most commonly used  
47 cereal grains growing all over the world and feeding more than 40% of the world population.  
48 Amongst the different types of wheat grain, bread wheat (*Triticum aestivum* L., AABBDD) is  
49 the most economically important crop and the world's most widely cultivated cereal. It is  
50 originated from hybridization between *Triticum urartu* (AA) and *Aegilops speltoides*-related  
51 species (BB), forming *Triticum turgidum* ssp. *dicoccoides*, and again hybridized between  
52 *Triticum turgidum* ssp. *durum* (AABB) and *Aegilops tauschii* (DD), forming the modern  
53 hexaploid bread wheat (AABBDD).

54 Resistant cultivars are often regarded as one of the most effective tools for controlling CCNs.  
55 Many sources have been reported and reviewed for conferring resistance measures. Important  
56 sources of resistance genes were revealed in landrace varieties by identifying many resistance  
57 *Cre* genes. In recent years, different types of molecular markers have been applied in plants  
58 such as Restriction fragment length polymorphisms (RFLPs), microsatellites or simple  
59 sequence repeats (SSRs), expressed sequence tags (ESTs), cleaved amplified polymorphic  
60 sequence (CAPS), randomly amplified polymorphic DNA (RAPD), amplified fragment length  
61 polymorphisms (AFLPs), inter simple sequence repeat (ISSR), Diversity arrays technology  
62 (DArT) and single nucleotide polymorphism (SNP) (Dhingani *et al.*, 2015). In genetic studies,  
63 single nucleotide polymorphisms (SNPs) are one of the most effective tools. SNPs are more  
64 powerful in estimating population structure which are abundant in the genome.

65 In recent years, research on wheat genome recorded 90K SNP by the new Infinium to 500K  
66 and 4 M in Illumina shotgun WGS array (Avni *et al.*, 2014; Wang *et al.*, 2017; Lai *et al.*,  
67 2015). Association mapping (AM), is known extremely for the identity of markers associated  
68 traits based on linkage disequilibrium (LD) in plants. AM has been applied to discovery of  
69 quantitative trait loci (QTL) on chromosomes in range of crop species. To date, QTL regions  
70 on different chromosomes were detected in association with particular traits using AM in wheat  
71 such as pre-harvest sprouting resistance, low  $\alpha$ -amylase and seed color (Rabieyan *et al.*, 2022)  
72 and grain-associated traits (Wang *et al.*, 2017), resistance to CCNs (*Heterodera* spp.),  
73 resistance to root lesion nematode (*Pratylenchus* spp.) and resistance to crown rot (Dababat *et*  
74 *al.*, 2016; Erginbas-Orakci *et al.*, 2018; Kumar *et al.*, 2021; Sohail *et al.*, 2022).

75 Several QTLs have been suggested to affect on resistance to *H. filipjevi*. The first survey of  
76 QTLs conferring resistance to *H. filipjevi* in wheat reported eleven QTLs on chromosomes  
77 1AL, 2AS, 2BL, 2D, 3AL, 3BL, 4AS, 4AL, 5BL, 6B, 6D and 7BL (Pariyar *et al.*, 2016;  
78 Dababat *et al.*, 2021).

79 The aim of the present study was to *a*: find marker-trait associations within 188 wheat  
80 genotypes collected from West Asia-North Africa, WANA, *b*: identify SNPs associated with  
81 resistance to *H. filipjevi* in wheat, *c*: combine analyses of phenotypic data and association  
82 mapping.

83

## 84 MATERIAL AND METHODS

### 85 Plant Materials and Inoculum Preparation

86 A total of 223 wheat accessions originating mostly from West Asia and North Africa (WANA  
87 countries) with three wheat cultivars as susceptible and resistant controls were used to evaluate  
88 their resistance to *H. filipjevi*. It is worth to note that 188 accessions out of 223 accessions used  
89 for phenotyping indicated sufficient DNA quality for SNP calls. The wheat accessions were  
90 provided by the International Center for Agricultural Research in the Dry Areas (ICARDA),  
91 and were originated from Afghanistan (7), China (1), Iran (164), Iraq (3), Morocco (1), Pakistan  
92 (7) and Syria (5) countries. The pedigree of the 188 wheat genotypes used in this study is given  
93 in supplementary Table.1. For the preparation of inoculum, the collecting of nematodes,  
94 extracting, identifying, incubation of the cysts and obtaining infective juveniles were conducted  
95 as described by Majd Taheri *et al.*, (2019).

96

### 97 Phenotyping Assessment

98 The phenotypic evaluation was performed in a growth chamber at the Iranian Research  
99 Institute of Plant Protection (IRIPP). Wheat seeds of each accession, were sterilized,  
100 germinated and planted in a plastic tube filled with a mixture of sand, field soil, and organic  
101 matter (70:29:1, v:v:v) arranged in a completely randomized design with five replications. The  
102 wheat cultivars Bezostaya and Sonmez were chosen as the susceptible and resistant checks,  
103 respectively. Each plant was inoculated with 1 mL of inoculum containing 500 fresh second  
104 stage juveniles in a water suspension. After nine weeks, the level of resistance was counted and  
105 categorized into four groups based on the number of white females and cysts, Resistant (R)  $\leq$   
106 3; Moderately resistant (MR) = 3–7; Susceptible (S) = 7–20; Highly Susceptible (HS)  $\geq$  20  
107 according to Sharma *et al.*, (2013). Normality of data and Homogeneity of variances were  
108 examined with Shapiro-Wilk test and Levine's test, respectively. All phenotypic data were

109 analysed using Generalized Linear Model (GLM) using statistical software SAS v9.4 and mean  
110 separation was conducted using Duncan's Multiple Range Test.

111

### 112 **Genotyping and Data Preprocessing**

113 Genomic DNA was extracted from fresh leaves using a modified CTAB  
114 (cetyltrimethylammonium bromide) method as described by Saghai-Marooft *et al.* (1984).  
115 Samples were genotyped by genotyping-by-sequencing (GBS) and Diversity Arrays  
116 Technology (DArT) (Sansaloni *et al.*, 2011) using 152K SNP panel at the Genetic Analysis  
117 Service for Agriculture (SAGA) at the International Maize and Wheat Improvement Center  
118 (CIMMYT), Mexico. The quality of genotypic data were curated by removing SNPs with  
119 minor allele frequency (MAF) less than 0.05 and missing data more than 20% from the  
120 subsequent analysis (Bhatta *et al.* 2018), and the heterozygous data were considered as missing  
121 data (Mourad *et al.* 2018; Pariyar *et al.* 2016), which left a set of 10,471 polymorphic SNP  
122 markers with known chromosomal position (based on Chinese spring map of IWGSC RefSeq  
123 v1.0 assembly (Appels *et al.*, 2018)) for further analysis.

124

### 125 **Analysis of Population Structure**

126 The 840 SNP markers were selected based on physical position on chromosomes (A, B and  
127 D) from the total 10,471 markers with known chromosomal positions. Population structure  
128 analysis was performed using a Bayesian model in software STRUCTURE v2.3.4 (Pritchard  
129 *et al.*, 2000), where number of populations (K) were assumed from 1 to 10 using 100,000 burn  
130 iterations followed by 100,000 Markov-Chain Monte Carlo (MCMC) iterations. Process was  
131 repeated 5 times for each K. Output was visualized using STRUCTURE harvester and the  
132 optimal K value was identified based on the LnP(D) and Evanno's  $\Delta K$  (Evanno *et al.*, 2005).

133

### 134 **Linkage Disequilibrium Association mapping**

135 Linkage disequilibrium and Genome-Wide Association Study, GWAS were implemented  
136 using 10,471 SNPs with known chromosomal positions. Chinese Spring genome map IWGSC  
137 RefSeq v1.0 assembly was used as the reference genome (Appels *et al.*, 2018). A mean pairwise  
138  $r$  for the 21 chromosomes was determined. The LD heat maps plot for significantly associated  
139 SNPs was constructed by using Haploview software 4.2 (Broad Institute, Cambridge, MA).  
140 GWAS was conducted using the General linear model (GLM) and Mixed linear model (MLM)  
141 (Q+K) in TASSEL v. 5.2.51 (Bradbury *et al.*, 2007). The Q matrix was adapted from the K=2  
142 for association mapping for controlling spurious results due to population stratification as a  
143 major issue in GWAS. TASSEL software was employed to estimate kinship matrix and the

144 association analyses were carried out to generate Manhattan and quantile-quantile plots (Q–Q  
145 plot). A threshold P-value of 0.001 ( $-\log_{10}P= 3$ ) was applied to declare significant SNPs for  
146 marker-trait association results. The phenotypic variation ( $R^2$ ) was estimated for significant  
147 markers. To reduce the false discovery rate, FDR was implemented at 0.001 level in SAS v 9.4  
148 (SAS Institute Inc., Cary, NC, United States).

## 149 150 **RESULTS AND DISCUSSION**

151 Wild relatives of wheat are important sources of disease resistance. In recent years, different  
152 types of molecular markers have been applied to study the genetic traits in many crops i.e.,  
153 barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), potato (*Solanum tuberosum* L.), rice (*Oryza*  
154 *sativa* L.), soybean (*Glycine max* (L.) Merr.), sorghum (*Sorghum bicolor* L.) Moench), tomato  
155 (*Lycopersicon esculentum* Mill.) and wheat (*Triticum aestivum* L.). SNP chips were mostly  
156 applied in GWAS which makes it easier to identify QTLs associated with certain traits. **Our**  
157 **raw data and variances were normal and homogeneous, respectively.** The analyses of  
158 phenotypic data revealed significant differences among the accessions for resistance to *H.*  
159 *filipjevi* (Table 1). The 35 % of wheat accessions showed resistant (R) reaction to *H. filipjevi*,  
160 44% of the accessions were moderately resistant (MR) and 21% were susceptible (S) (Figure  
161 1). Most of the Iranian genotypes indicated moderately resistant (45%) trait (Majd Taheri *et*  
162 *al.*, 2019). Of the 10,471 SNPs found to be highly associated with resistance to *H. filipjevi*,  
163 4,096 (39%), 4,739 (45%), and 1,636 (16%) SNPs were recorded on the AA, BB, and DD  
164 genomes, respectively (Figure 2). **AA and BB genomes have a higher distribution of SNPs than**  
165 **the DD genome, this finding is in agreement with similar studies (Wen *et al.*, 2017; Gahlaut *et***  
166 ***al.*, 2019; Rabieyan *et al.*, 2022; Tehseen *et al.*, 2022).** The minimum number of SNPs were  
167 associated with resistance to *H. filipjevi* from chromosome 4D (147 SNPs) and most numbers  
168 of SNPs were from 2B (887 SNPs). Population structure analysis implemented using 840  
169 markers, indicated two possible subpopulations, based on the clear peak at  $k=2$  (Figure 3). The  
170 first and second group consisted of 62% and 38% of the wheat accessions, respectively. We  
171 found significant differences among the genotypes for resistance to *H. filipjevi*. The genetic  
172 diversity of wheat genotypes from our previous experiment revealed the suitability of this  
173 group of wheat genotypes for association mapping studies (Majd Taheri *et al.*, 2019).

174 Using 10,471 SNPs, linkage disequilibrium (LD) was determined by calculating squared  
175 correlation coefficient ( $r^2$ ) for the 21 chromosomes. **We applied a mixed linear model (MLM)**  
176 **and General linear model (GLM) in GWAS analysis.** QQ-plots and Manhattan plots of the  
177 GWAS results of both GLM and MLM analysis were compared for resistance trait which are

178 shown in Figure 4. Based on the obtained QQ-plot from GLM and MLM models, the Q-Q plot  
179 of GLM shows deviations from the slope line, demonstrating the loci which deviate from the  
180 null hypotheses and indicating significant positive marker-trait association which makes GLM  
181 as a better approach. Manhattan plots represent the profile of the P-value of SNPs in Figure 5.  
182 A total of 11 SNPs significantly associated with resistance to *H. filipjevi* trait and crossed the  
183 false detection rate (FDR) at  $p < 0.001$  were identified. The phenotypic variation ( $r^2$ ) explained  
184 by the individual SNPs ranged from 7 to 13% (Table 2).

185 So far, some significant Marker-trait associations (MTAs) were identified on wheat  
186 chromosomes to agronomic characteristics and diseases. This collection of wheat genotypes  
187 has not been utilized for resistance studies to cereal cyst nematode so far, however, GWAS of  
188 diverse panels against *H. filipjevi* was done by Pariyar *et al.*, (2016) and Dababat *et al.*, (2021).  
189 In the present study, 11 markers were significantly ( $p$ -value  $< 0.001$ ) associating with resistance  
190 to *H. filipjevi* which were detected on chromosomes No 2A, 3B, 4A, 4B, 5A, 5B, 5D and 6B.  
191 The linkage disequilibrium (LD) analysis for all significantly associated SNPs showed that 3  
192 markers on 4A and 2 markers on 4B Chromosomes were in high intra-chromosomal LD, hence  
193 the 11 SNPs could be reduced to 8. It is noteworthy that the D genome carries only one of all  
194 identified MTAs in this study, likely implies the low level of diversity in the D genome  
195 originated from the late hybridization of *Aegilops tauschii* during the evolution of common  
196 wheat (Gahlaut *et al.*, 2019). A previous GWAS have demonstrated 11 QTLs on chromosomes  
197 1AL, 2AS, 2BL, 3AL, 3BL, 4AS, 4AL, 5BL and 7BL (Pariyar *et al.*, 2016). Another study  
198 identified QTLs on chromosomes 1A, 2A, 2B, 2D, 3A, 6B, and 6D were detected using a mixed  
199 linear model (MLM) (Dababat *et al.*, 2021). Fourteen genes for resistance to CCN have been  
200 identified which include the following: *Cre1*, *Cre2*, *Cre3*, *Cre4*, *Cre5*, *Cre6*, *Cre7*, *Cre8*, *Cre9*,  
201 *CreR*, *CreV*, *CreX*, *CreY* and *CreZ* (Ali *et al.*, 2019; Kishii, 2019; Dababat *et al.*, 2021). CCN  
202 resistance genes *Cre1*, *Cre2*, *Cre3*, *Cre5* (syn. *CreX*), *Cre6*, *Cre8* and *CreR* identified in wheat  
203 and its relatives on chromosome 2B, 2A, 2D, 2A, 5A, 6B and 6D, respectively (Slootmaker *et*  
204 *al.*, 1974; Asiedu *et al.*, 1990; Eastwood *et al.*, 1991; Delibes *et al.*, 1993; Jahier *et al.*, 1996;  
205 Paull *et al.*, 1998; Ogonnaya *et al.*, 2001). Our results demonstrated three QTLs (on 2A, 5A  
206 and 6B) found on chromosomes with identified resistance genes. Surprisingly *Cre8* gene as a  
207 resistance gene to CCN, *H. avenae* was mapped by Williams *et al.*, (2003) on chromosome 6B,  
208 moreover the effective role of *Cre8* in conferring of resistance to CCN, *H. filipjevi* in wheat  
209 was emphasized by Imren *et al.*, (2013). Our finding suggests that the marker identified in this  
210 study may be present in the genomic region of the *Cre8* gene, however further evidences are  
211 needed to confirm the exact loci. Similar to the present study some QTLs that confer resistance



212 against other cereal nematodes were recently reported on wheat, *i.e.* in *H. avenae* on  
213 chromosomes 5A, 5B, 5D and 6B, in root lesion nematodes, *P. neglectus* on chromosomes 3B,  
214 4A and 6B, *P. thornei* on chromosomes 2A, 3B and 5B (Dababat *et al.*, 2016).

215 Pleiotropic effect resistance genes to multiple races (HG types) of soybean cyst nematode  
216 (SCN), *H. glycines* and Root knot Nematode (*Meloidogyne incognita*) was revealed in soybean  
217 line 438489B which carrying multi-nematode resistance gene package (Vuong *et al.*, 2011).  
218 Recently two QT controlling reniform nematode, RN (*Rotylenchulus reniformis*) resistance  
219 were identified in the SCN resistance gene *GmSNAP18* at the *rhg1* locus and its paralog  
220 *GmSNAP11* in soybean line 438489B (Usovsky *et al.* 2021). Hence, there is a possibility of  
221 similarities between identified QTLs which necessitates further experiments to determine  
222 common QTL between two or more pathogens. Importantly, it is obvious that a QTL with  
223 capability of inducing resistance to plant against several traits, is a valuable resource in  
224 breeding programs. In conclusion, in this study, 188 wheat accessions were applied to perform  
225 association mapping, and 10,471 SNPs used for GWAS after filtering according to the MAF,  
226 missing data, and heterozygous data. We estimated the phenotypic and genotypic parameters  
227 for resistance trait and eleven significantly associated SNP markers were detected by GLM.  
228 Based on the results, the use of populations from different genetic backgrounds provide further  
229 progress in identifying valid QTLs. The findings of present study demonstrated valuable  
230 sources of resistance in the studied wheat genotypes to a widespread and important species of  
231 CCN in some areas of the crescent fertile region for inclusion in future breeding programs by  
232 new resistance gene resources.

233

## 234 ACKNOWLEDGEMENTS

235 We are particularly grateful to the Iranian Research Institute of Plant Protection (IRIPP),  
236 International Center for Agricultural Research in the Dry Areas (ICARDA) and International  
237 Maize and Wheat Improvement Center (CIMMYT) for valuable supports.

238

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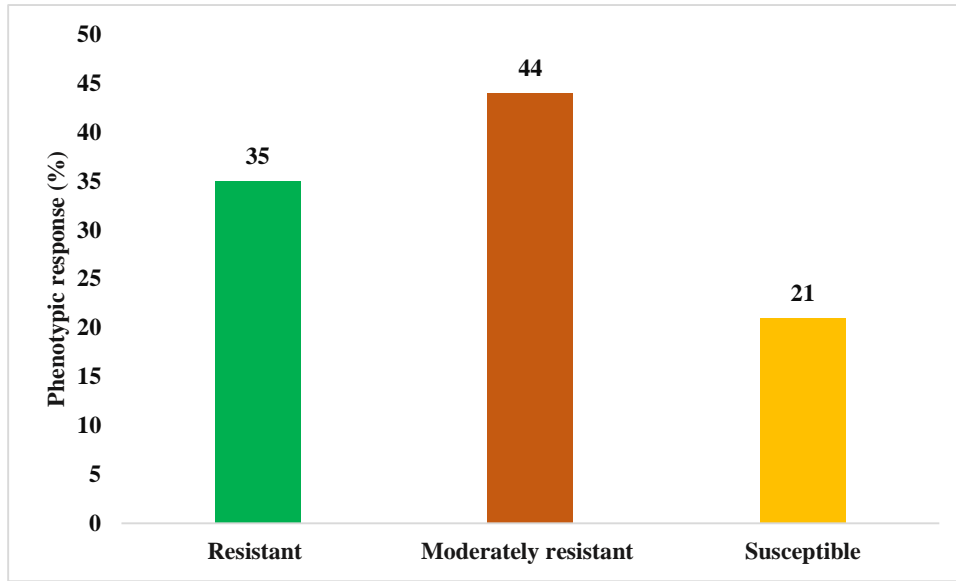
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385 **Fig 1.** Phenotypic responses of wheat accessions to *Heterodera filipjevi* based on the number  
 386 of white females and cysts (Resistant $\leq$  3; Moderately resistant= 3-7; Susceptible= 7-20).

387  
 388 **Table 1.** Analysis of variance of the reaction of wheat genotypes to *Heterodera filipjevi* using  
 389 Generalized Linear Model (GLM).

Source	Degrees of freedom	Mean of square	F value	Pr > F
Genotype	225	1.15	3.60	<0.0001
Error	904	0.32	-	-
CV <sup>a</sup>	-	25.09	-	-

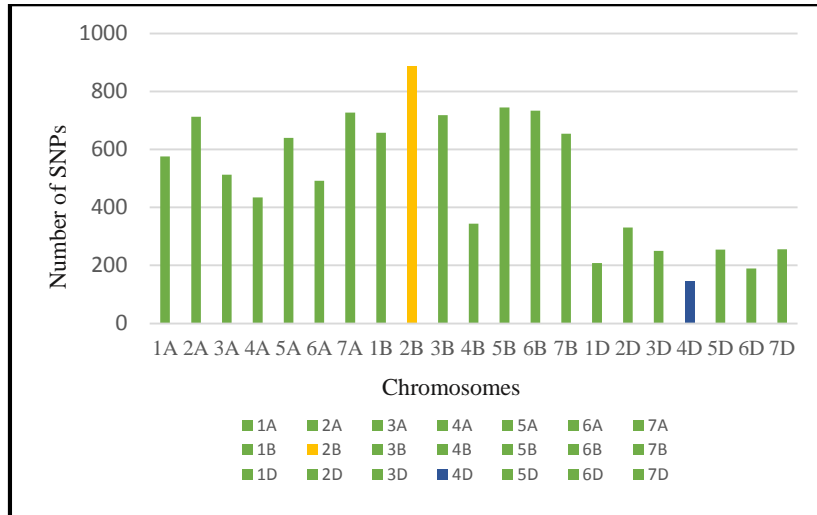
390 <sup>a</sup> Coefficient of variation.

391  
 392 **Table 2.** Single nucleotide polymorphisms (SNPs) significantly associated with resistance to  
 393 *Heterodera filipjevi*.

No.	SNP Marker	CHR	POS (bp)	FDR	P value	Allele	Allelic effect	R <sup>2</sup> (%)	cM
1	3034005	2A	7919418	0.00060	0.00007	T/C	-0.49	10	8
2	1106119	3B	183514671	0.00070	0.00034	T/G	0.89	13	-
3	2262587	4A	374449717	0.00060	0.00017	C/T	0.42	10	-
4	1220611	4A	430870112	0.00070	0.00046	T/C	-0.41	8	-
5	2266236	4A	433757511	0.00070	0.00037	G/A	-0.40	8	27
6	1128101	4B	605941582	0.00100	0.00081	G/A	-0.49	8	-
7	1244896	4B	608261318	0.00060	0.00023	C/G	0.52	9	45
8	1098989	5A	480788416	0.00100	0.00097	G/C	0.57	7	51
9	1209179	5B	506087964	0.00060	0.00011	A/G	-0.91	9	48
10	2260283	5D	380840282	0.00100	0.00096	G/A	0.52	7	-
11	1091272	6B	79045627	0.00070	0.00051	A/G	-0.42	8	23

394 **CHR:** Chromosome; **POS:** Position; **FDR:** False Discovery Rate; **R<sup>2</sup>:** Effect due to genetic variation; **Cm:**  
 395 **Centimorgan.**

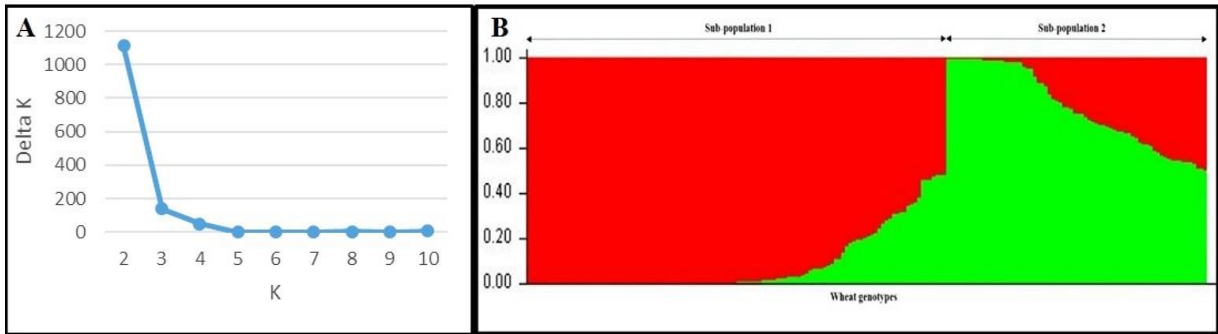
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398 **Fig 2.** Genome origin (A, B and D) of tested wheat SNPs of tested wheat genotypes. **Yellow**  
 399 **and blue columns represent highest and lowest numbers of SNPs, respectively.**

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402 **Fig 3.** A: Graph of delta K values showing highest probability at number of groups (K= 2) and  
 403 B: Estimated population structure of 188 wheat genotypes on k= 2.

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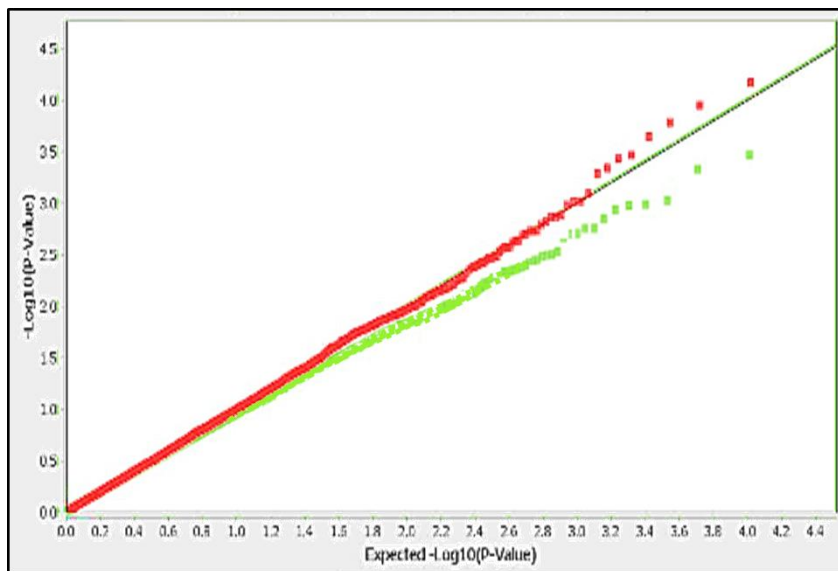
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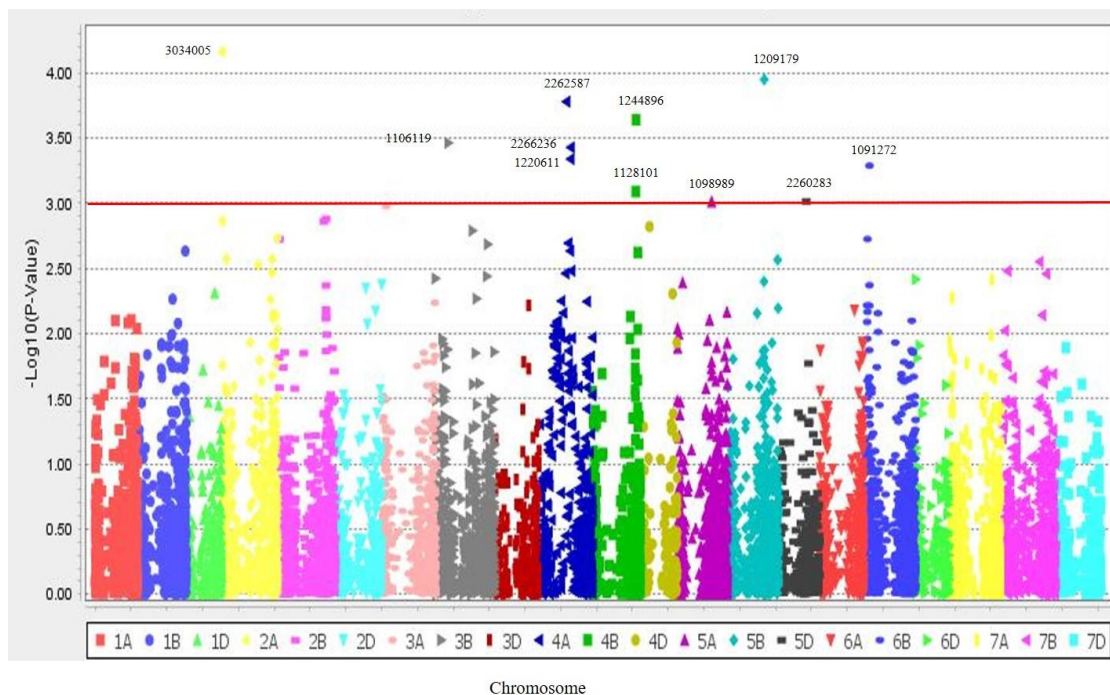
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421 **Fig 4.** QQ (Quantile-Quantile) plots, Red line represents the observed P values using the GLM  
 422 (Q) model and green line represents the observed P values using the MLM (Q + K) model.

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425 **Fig 5.** Manhattan plots of  $P$  values showing genomic region of wheat genotypes associated with *Heterodera filipjevi* resistance. The X-axis represents the position of markers over the  
 426 wheat chromosomes and Y-axis represents  $-\log_{10}$  (P-values) of the marker-trait association.  
 427 Each Point in the plot represents a SNP marker. The red line represents the threshold for  
 428 genome-wide significance. Markers with  $-\log_{10}$  (P-values) above the threshold are candidates.  
 429  
 430

431 **شناسایی آل‌های SNP مرتبط با مقاومت گندم به نماتد سیستمی غلات *Heterodera filipjevi* با**  
 432 **استفاده از نقشه یابی ارتباطی گسترده ژنوم**

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#### چکیده

435

436 وجود صفت مقاومت در گیاهان از لحاظ دسترسی به ژرم پلاسماهای مقاوم امید بخش حائز اهمیت اقتصادی است. این  
 437 مطالعه به منظور بررسی ارتباط نشانگر- صفت در تعداد زیادی از جمعیت‌های گندم نان نسبت به نماتد سیستمی غلات  
 438 *Heterodera filipjevi* انجام شد. نتایج حاصل از تجزیه و تحلیل آماری داده‌های فنوتیپی با استفاده از مدل خطی تعمیم  
 439 یافته (GLM) نشان داد، ژنوتیپ‌ها از لحاظ واکنش مقاومت به نماتد از اختلاف معنی‌داری برخوردار هستند. ارزیابی  
 440 ژنوتیپی با استفاده از یک تراشه K SNP 152 صورت گرفت. پس از اعمال کنترل کیفیت روی مجموعه داده-ها، تعداد  
 441 10471 نشانگر SNP برای نقشه‌یابی ارتباطی گسترده ژنوم (GWAM) استفاده شد. آنالیز ساختار جمعیت با استفاده از  
 442 840 نشانگر SNP، جمعیت مورد مطالعه را به دو زیر جمعیت طبقه بندی نمود. یازده نشانگر متعلق به هشت جایگاه  
 443 ژنی به طور معنی داری ( $p$ -value < 0/001) در ارتباط با صفت مقاومت به نماتد روی کروموزوم-های A2، 3B،  
 444 4A، 4B، 5A، 5B، 5D و 6B شناسایی شدند. از میان 11 نشانگر شناسایی شده، سه نشانگر روی کروموزوم 4A  
 445 دو نشانگر روی کروموزوم 4B از میزان عدم تعادل پیوستگی بالایی برخوردار بودند. لذا تعداد 11 نشانگر شناسایی  
 446 شده، به هشت نشانگر کاهش یافت. مطالعه حاضر منابع ارزشمندی از مقاومت به نماتد سیستمی غلات را در ژنوتیپ‌های  
 گندم نشان داد. نشانگرهای مرتبط را می‌توان در برنامه‌های اصلاح مولکولی گندم نان استفاده کرد.