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Marker-trait association of Russian wheat aphid (*Diuraphis noxia*) resistance in a globally diverse set of wild barley

Original paper

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Abstract: The Russian wheat aphid (RWA, *Diuraphis noxia*), prevalent pest affecting barley globally, can severely damage barley yield and grain quality. The present study aimed to identify simple sequence repeat (SSR) markers associated with RWA resistance in wild barley (*Hordeum vulgare* subsp. *spontaneum*). Thirty wild barley accessions were selected from the ICARDA GeneBank and Iran to represent 10 Asia countries scattered along the natural geographic distribution of the species for RWA resistance. Five traits, including leaf chlorosis, leaf rolling, chlorophyll a, b, and total chlorophyll concentration, were evaluated. Fourteen SSR markers were used to assess genetic diversity. The association between 52 polymorphic SSR markers and RWA resistance traits was examined using both general linear model (GLM) and mixed linear model (MLM) approaches. The population structure analysis revealed seven subpopulations in the entire collection (K=7). Eleven marker-trait associations (MTA) were identified on chromosomes 1H, 2H, 3H, 4H, and 7H, with significant associations found for markers Bmag007, Bmag6, Bmac0399, Bmag0125, and EBmac0701 with RWA resistance traits in both GLM and MLM. This study identified novel genomic regions putatively linked with RWA resistance traits, offering insights into the genetic architecture of RWA resistance in barley. These findings may guide the utilization of RWA resistant accessions of *H. spontaneum* as parents for barley breeding programs.

Keywords: association analysis, seedling resistance, SSR marker, subpopulations.

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Introduction

The Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (*Hemiptera aphididae*), is a significant pest affecting two important cereal crops: wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.). Russian wheat aphids (RWA) cause a significant loss in grain yield and quality in barley. As a phloem-feeding aphid, it causes symptoms such as leaf rolling and streaking, head trapping, and the eventual death death of the infested plants (Nieto Lopez and Blake, 1994). RWA infestation decreases plant height, shoot weight, and number of spikes (Gharaghanipor et al., 2022).

Hordeum vulgare ssp. *spontaneum* is the direct progenitor of domesticated barley, the only wild species in the barley primary gene pool and hence readily crossable species (Zhang et al., 2017). Genetic diversity of *H. spontaneum* has been estimated to be twice that of cultivated barley (Able et al., 2007). Many resistance attributes to diseases and pests have been lost during domestication process in the wild relatives of cultivated crops (Moreira et al., 2018). Screening crop wild relatives for (partial) resistance to aphids supports a fine chance for identifying potentially beneficial traits (Zohary and Hopf, 2000) and introducing them into agricultural cultivars (Webster et al., 1991; Arora et al., 2019).

Exploring new sources of resistance and understanding of complex genetic components involved in pest resistance is necessary to develop pest resistant cultivars. Marker assistant selection (MAS) may be employed efficiently with DNA markers strongly related to target loci and highly polymorphic, allowing for low-cost recognition of RWA resistant plants (Pritchard et al., 2000; Collard and Mackill, 2008; Joukhadar et al., 2013). Scanty research has focused on association mapping of RWA resistance (Heng-Moss et al., 2003; Dahleen et al., 2012; Joukhadar et al., 2013; Tolmay et al., 2013; Dahleen et al., 2015; Zhang et al., 2017). Resistance to RWA in barley was associated with loci on chromosomes 1H, 2H, 3H, 5H, and 7H (Dahleen et al., 2012). A total of 605 high-quality bin-mapped SNPs were positioned in seven linkage groups with a total map length of 1208.9 cM and average marker distance of 2.02 cM. Composite interval mapping identified five different quantitative trait loci (QTLs) on chromosomes 1H, 3H, 5H and 6H associated with aphid resistance and related traits (Rohlf, 1988). The novel chromosomal regions that could contribute to RWA resistance in wheat were found, and the intricate genetics that could control RWA resistance were uncovered (Kisten et al., 2020). Macaulay et al. (2020) proved an aphid resistance site on the distal fragment of the 2HS chromosome, with resistance inherited from the previously identified *H. v.* ssp. *spontaneum* (Ahman and Bengtsson, 2019).

Association genetics or association mapping (AM) is one of the approaches that is currently being used because of its multiple applications to many crops. Association genetics is useful as a novel strategy for discovery of new markers to use in marker-assisted selection (MAS) and breeding or for confirmation of quantitative trait loci (QTL) (Alvarez et al., 2014). Because of its ability to use the natural diversity and to search for functional variants in a broader germplasm, association mapping is becoming popular among researchers (Kisten et al., 2020).

The present study was conducted to evaluate a diverse set of *H. spontaneum* accessions for RWA resistance and identification of SSR markers associated with RWA resistance by association analysis.

Materials and Methods

Plant materials

Thirty natural accessions of wild barley (*H. spontaneum*), 27 accessions provided from International Center for Agricultural Research in Dry Areas (ICARDA) GeneBank representing nine countries scattered along the natural geographic distribution of the species and three genotypes collected from the west and north-west of Iran, were evaluated (Table 1).

Phenotyping

Plant growth**:** The plant seeds were sterilized in 2% (v/v) hypochlorite and rinsed with distilled H_2O. Afterwards, they were incubated at 4°C for 14 days. Then they were kept at room temperature for 48 h. The pots were filled with a mixture of soil, sand, gravel, peat moss and perlite in a ratio of 1: 1: 1: 1: 3, respectively. The germinated seedlings were grown in controlled greenhouse conditions with a

temperature of 20° C during the day, 15° C at night, 16 hours of light per day and 8 hours at night.
The germinated seedlings were grown in controlled greenhouse conditions with a temperature of 20° C during the day, 15 ° C at night, 16 hours of light per day and 8 hours at night. Aphid tests**:** *D. noxia* was collected from wheat fields near Ilam (Iran).

Table 1. Origin of wild barley accessions used in this study.

No.	Accession number	Collection location		Latitude	Longitude
		Province	Country		
1	IG 120790	Krasnvvdsk	Turkmenistan	N38 25 55	E056 16 44
2	IG 38654	Faryab	Afghanistan	N35 44	E063 59
3	IG 38669	Samangan	Afghanistan	N36 16	E068 01
4	IG 40155	Kashkadrya	Uzbekistan	N38 57	E66 50
5	IG 117888	Aydlyb	Syria	N36 12 30	E36 46 38
6	IG 40154	Kashkadrya	Uzbekistan	N38 48	E66 28
7	IG 39919	Svydya	Syria	N32 36 00	E36 44 30
8	IG 120794	Ashgabat	Turkmenistan	N38 35	E57 07
9	IG 39565	West Bank	Palestine	N31 46 20	E35 15 37
10	IG 140189	Kulyab	Tajikistan	37.82496	70.18093
11	IG 144170	Alsalt	Jordan	N32.10835	E35.74527
12	IG 137596	Arart	Armenia	N39 47	E45 22
13	IG 120793	Ashgabat	Turkmenistan	N38 30	E56 50
14	IG 129152	Nineveh	Iraq	N36 21	E43 08
15	IG 38936	West Bank	Palestine	N32 24	E35 06
16	IG 142356	Svghad	Tajikistan	N39 48 04	E68 53 35
17	IG 119447	Aydlyb	Syria	N35 32 45	E37 01 20
18	IG 38657	Kandahar	Afghanistan	N31 40	E65 29
19	IG 121853	Svydya	Syria	N32 37 30	E36 46 25
20	IG 139141	Alkrak	Jordan	N31 19.786	E35 43.341
21	IG 142486	Svghad	Tajikistan	N40 07 30	E69 13 57
22	IG 119438	Hvms	Syria	N35 00 48	E36 42 09
23	IG 40152	Svrkhandarya	Uzbekistan	N37 48	E67 00
24	IG 140352	Kulyab	Tajikistan	38.25927	69.83140
25	IG 39489		Syria	N35 00	E038 00
26	IG 140073	Dushanbe	Tajikistan	38.37815	68.70809
27	IG 140302	Kulyab	Tajikistan	38.09862	69.79325
28	IG1	Kurdistan	Iran	N35 0 55	E46 57 52
29	IG2	Kermanshah	Iran	N24 32 08	E48 01 39
30	IG3	Kermanshah	Iran	N24 25 01	E47 02 44

Experiments were conducted in a greenhouse on susceptible 'Sardari' barley plants at Ilam University. The identity of *D. noxia* biotype 2 was confirmed in diagnostic barley differential

greenhouse assays. At the stage of two to four leaves, five aphids were used to infest each plant. The aphid needed for infestation was collected from the wheat fields in Ilam province and transferred to the incubator with the conditions of temperature 25±1, humidity 85%, 14 hours of light and 10 hours of darkness (Girma et al., 1993). In order to achieve the required number of aphids to infest plants and create a pure population, aphids were propagated on the sensitive barley variety (Sarroud). The infestation of the population was conducted using five aphid nymphs aged 4-5 were released on each seedling. Twenty eight days after infestion, reaction to RWA infestation of *H. spontaneum* accessions was recorded by six replicates with five seedlings tested per accession within each replicate (Tocho et al., 2012).

All Plants were visually scored for leaf chlorosis and leaf rolling. Chlorosis was recorded on a scale of 0–9 (Tolmay et al., 2020), where 0 is immune, 1 represents healthy appearing plant, 2 denotes prominent isolated chlorotic spots, and 9 represents dead or damaged beyond recovery. Leaf rolling was recorded using a 1–3 scale (Tolmay et al., 2020) where 1 showed unfolded, 2 indicates one or more folded leaves, and 3 indicates one or more ring-shaped folded leaves.

Chlorophyll concentration

To measure the amount of chlorophyll a, b and total chlorophyll, about 0.5g of control and aphid-infested plant leaves were ground with liquid nitrogen. Five ml of the 80% acetone was added up to extract photosynthetic pigments (chlorophyll a, b, and total). Approximately 1.5 ml of the blend was aspirated and then centrifuged at 3000 rpm for 15 min to remove unsolvable plant tissues. In order to guarantee the absorbance readings at 663 nm below 1.5, the resultant supernatant was diluted with 80 % acetone. Next, pigment extract absorbance was calculated at 646 nm and 663 nm wave lengths, respectively in a spectrophotometer (Cary-50 model made by Varian Company, Australia). Three types of pigment concentrations were determined according to equation bellow 6:

$$C_a \text{ (mg gr}^{-1}) = 12.21\ A_{663} - 2.81\ A_{646}$$
$$C_b \text{ (mg gr}^{-1}) = 20.13\ A_{646} - 5.03\ A_{663}$$
$$Ct \text{ (mg gr}^{-1}) = Ca + Cb$$

In these equations, Ca, Cb, and Ct represent the chlorophyll a, b, and total concentrations, respectively. A_x represents the x nm absorbance.

Genotyping

DNA extraction was done at the plant stage of three to five leaves 34. PCR mixture was comprised of 40-50 ng/µl genomic DNA, 1 mM deoxyribonucleotide triphosphates (dNTPs), 2 mM $MgCl_2$, 19 PCR buffer, 1.5 U *Taq* DNA polymerase (Sina clone Company, Karaj, Iran), and 0.7 µM of forward and reverse primers reported by Raman and Read (2000) (Table 2) in a total volume of 20 µl. The PCR reaction was performed using the protocol of Cakir et al. (2009). The amplified products were resolved on a non-denatured polyacrylamide gel at a concentration of 6%. The electrophoresed gels were visualized under UV light.

Statistical analysis

The analysis of variance was was conducted using a completely randomized design, and genotypic means were compared using Duncan's multiple range post-hoc tests in SAS version 9.2.

Polymorphic information content (PIC) indicates the genetic diversity of primers. The PIC for each primer was determined by the following equation (Moreira et al., 2018):

$$PIC = 1-\Sigma\ (P_{ij})^2$$

In this equation, P_{ij} is the frequency of j^{th} allele in i^{th} primer.

In order to calculate genetic relationships among the wild barley accessions NTSYSpc 2.02e software was used for computing Jaccard's similarity coefficients. Furthermore, cluster analysis was conducted utilizing an unweighted pair-group (UPGMA) approach to develop dendrograms 39 using th above software. Genome-wide association analyses were carried out using the general linear model (GLM) and mixed linear model (MLM) approaches in TASSEL 4.0 and the association between each RWA resistance related traits and the SSR markers was tested (Bradbury et al., 2007). The relevant Q values were used as covariates in GLM and MLM analyses. Manhattan plots showed the relationship between a phenotypic trait and a SSR marker ($P \leq 0.05$). The population structure of the wild barley accessions was determined by the Bayesian clustering method using STRUCTURE version 2.3.4 (Oliver et al., 2010).

Table 2. Detailed information on SSR markers used in the present study.

GenBank	Primer location	Melting temperature (°C)	Product size (bp)	Primer sequence (5'–3')
Bmac0399	1H	60	145	R:CGATGCTTTACTATGAGAGGT F:GGGTCTGAAGCCTGAAC
Bmac0032	1H	60	215	R:CCATCAAAGTCCGGCTAG F: GTCGGGCCTCATACTGAC
Bmac0134	2H	55	148	R:CCAACTGAGTCGATCTCG F: CTTCGTTGCTTCTCTACCTT
Bmag0125	2H	55	134	R:AATTAGCGAGAACAAAATCAC F:AGATAACGATGCACCACC
Bmac0067	3H	55	171	R:AACGTACGAGCTCTTTTTCTA F:ATGCCAACTGCTTGTTTAG
Bmag0006	3H	58	174	R:TTAAACCCCCCCCCTCTAG F:TGCAGTTACTATCGCTGATTTAGC
EBmac0701	4H	55	149	R:ATGATGAGAACTCTTCACCC F: TGGCACTAAAGCAAAAGAC
Bmac0310	4H	55	176	R:CTACCTCTGAGATATCATGCC F:ATCTAGTGTGTGTTGCTTCCT
Bmag0223	5H	58	127	R:TTAGTCACCCTCAACGGT F: CCCCTAACTGCTGTGATG
GBM1483	5H	55	150	R:CAGTGATATGGACTACGGCG F:CTTGTTCTCCACCTCGAAGC
EBmac602	6H	58	205	R:GATTGGAGCTTCGGATCAC F:CCGTCTAGGGAGAGGTTCTC
Bmag0173	6H	58	150	R:CATTTTTGTTGGTGACGG F:ATAATGGCGGGAGAGACA
Bmag0007	7H	58	185	R:TGAAGGAAGAATAAACAACCAACA F:TCCCCTATTATAGTGACGGTGTG
Bmag0135	7H	58	161	R:ACGAAAGAGTTACAACGGATA F: GTTTACCACAGATCTACAGGTG

Results

Phenotypic evaluation

The reaction of 30 wild barley genotypes to RWA feeding was evaluated. The results of the analysis of variation showed highly significant genotypic effects for rolling ($F = 160.41$, $df_t = 29$, $df_e = 720$, $P < 0.0001$) and chlorosis ($F = 22.12$, $df_t = 29$, $df_e = 720$, $P < 0.0001$).

A wide range of resistance to susceptibility was observed for leaf chlorosis with the average of 1.06 to 9. Mean reactions ranged from 1.03 to 3 for leaf rolling. Regarding chlorosis, IG140073 and IG120794 genotypes had the scores of 1.06 and 1.37, respectively, which categorize as resistant. IG129152 and IG117888 were susceptible with the scores 9 and 8.82, respectively (Table 3). IG2 (1.03) and IG120794 (1.04) had the least leaf rolling and were the most resistant genotypes. On the other hand, IG3 (3.00) was a moderately susceptible (Table 3).

Chlorophyll concentration

Chlorophyll a ($F = 3.2$, $df_t = 29$, $df_e = 60$, $P < 0.001$), b ($F = 3.54$, $df_t = 29$, $df_e = 720$, $P < 0.001$), and total

chlorophyll (F = 3.45, df_t =29, df_e = 720, P <0.001) concentrations were significantly different among the RWA-infested *H. spontaneum* populations. Chlorophyll a, b, and total chlorophyll concentrations were decreased significantly (0.88, 0.62 and 1.49 mg/g, respectively) on RWA-infested *H. spontaneum* populations when compared with those of the uninfested plants (1.08, 0.80 and 1.61 mg/g). In the RWA-infested condition, IG40154 genotype had the highest chlorophyll a (1.56 mg/g), b (1.159 mg/g), and total (2.72 mg/g). Additionally, IG129152 genotype exhibited the lowest levels of chlorophyll a (0 mg/g), chlorophyll b (0 mg/g), and total chlorophyll concentration (0 mg/g) (Table 4).

Table 3. Mean comparison of leaf chlorosis and leaf rolling in wild barley accessions under infestation with Russian wheat aphid at seedling stage.

Accessions	Plant chlorosis	Leaf rolling
IG129152	9.00 a	1.58 j
IG117888	8.82 a	2.89 cd
IG144170	7.93 b	2.89 d
IG38657	6.54 c	2.51 g
IG121853	5.95 d	2.54 g
IG119447	5.25 e	1.80 j
IG39919	5.17 e	2.82 d
IG38654	5.16 e	2.80 de
IG137596	4.35 f	2.93bc
IG139141	3.75 g	2.00 h
IG120790	3.69 gh	2.95 b
IG120793	3.44 ghi	2.03 g
IG1	3.40 ghi	1.85 i
IG142486	3.38 ghi	1.83 i
IG39489	3.29 hi	2.02 h
IG119438	3.21 i	2.6 ef
IG38936	3.14 i	2.54 ef
IG140352	2.72 j	2.02 h
IG140302	2.68 j	2.58 fg
IG2	2.45 jk	1.03 k
IG140189	2.44 jk	1.89 i
IG39565	2.31 jk	1.54 g
IG40155	2.16 k	1.41 j
IG38669	2.15 k	1.57 i
IG3	2.10 k	3.00 a
IG40152	2.06 k	1.49 j
IG142356	1.60 l	1.08 k
IG40154	1.46 l	1.44 j
IG120794	1.37 lm	1.04 k
IG140073	1.06 m	1.07k

*In each column, the means with at least one similar letter do not differ significantly at the 5% probability level.

Screening of SSR marker and assessment of genetic diversity

The system of SSR markers was used for analyzing the genetic diversity of 30 wild barley genotypes. Out of 14 microsatellite primers used, 10 primers (71%) showed acceptable polymorphism. Four pairs of primers (Bmag0135, Bmac0032, GBM1483 and Bmag0173) were excluded from further analysis due to the production of non-specific bands (Table 5). A total of 52 alleles were detected. Number of observed alleles ranged 4 to 8 with an average number of 5.2 alleles per locus. Bmag007 marker had eight alleles while Bmac0399, Bmag0125, Bmag0223, and EBmac602 had 4 alleles (Table 5). Moreover, at the DNA level, 100 percent of the amplification products showed polymorphism, indicating substantial diversity among *H. spontaneum* accessions (Table 5).

Table 4. Mean comparison of Chlorophyll a, b and total Chlorophyll in barley accessions under infestation with Russian wheat aphid at seedling stage.

Accession	Chlorophyll b	Chlorophyll a	Total chlorophyll
IG40154	1.159a	1.56 a	2.72a
IG140073	0.96ab	1.39ab	2.35ab
IG2	0.957 ab	1.28a-d	2.23a-c
IG1	0.883a-c	1.34 a-c	2.23a-c
IG120790	0.819a-d	1.17a-d	1.986a-d
IG38936	0.816 a-d	1.14a-e	1.947a-d
IG38669	0.782b-d	1.13a-e	1.914a-d
IG140189	0.741b-d	1.09a-e	1.833b-d
IG40152	0.739b-d	1.081a-f	1.819b-d
IG3	0.725b-e	1.073a-f	1.798b-e
IG139141	0.706b-e	1.012b-f	1.72 b-e
IG39919	0.698b-e	1.015b-f	1.71 b-e
IG120794	0.696b-e	0.978b-f	1.67 b-e
IG142356	0.686b-e	0.988b-f	1.68 b-e
IG120793	0.679b-f	0.928 b-f	1.61 b-e
IG40155	0.617b-f	0.877b-g	1.49 b-f
IG38654	0.615b-f	0.897b-g	1.52 b-f
IG39489	0.599b-f	0.847c-g	1.45c-f
IG119438	0.571 c-g	0.802c-h	1.37c-g
IG140352	0.570 c-g	0.802c-h	1.37 c-g
IG140302	0.565c-g	0.760d-h	1.32 c-g
IG137596	0.519c-h	0.785d-h	1.305d-g
IG142486	0.496d-h	0.675 e-i	1.17d-h
IG39565	0.476d-h	0.658e-i	1.13d-h
IG119447	0.442 d-h	0.653e-i	1.09d-h
IG121853	0.353e-h	0.550f-i	0.903e-h
IG38657	0.309f-i	0.391 g-j	0.701 f-i
IG117888	0.228g-i	0.285h-j	0.514g-i
IG144170	0.165hi	0.191ij	0.356hi
IG129152	0.000l	0.000j	0.000l

*In each column, the means with at least one similar letter do not differ significantly from each other at the 5% level.

The highest and lowest PIC was for primer Bmag007 (0.812) and Bmac0399 (0.374), respectively. As a result, primer Bmag007 is an efficient and helpful marker for detecting genetic variations amongst *H. spontaneum* accessions (Table 5). According to the cluster analysis, the thirty accessions were divided into seven groups (Figure 1).

Table 5. Allele number (Na), number of effective alleles, marker index, Shannon index and polymorphism information content recorded in 30 wild barley accessions using 10 SSR markers.

Primer name	Number of observed alleles	Polymorphism information content (PIC)	Number of effective alleles	Marker index	Shannon index
Bmac0399	4	0.374	1.60	1.50	0.72
Bmac134	5	0.746	3.93	3.73	1.48
Bmag0125	4	0.470	1.88	1.88	0.76
Bmac0067	5	0.601hg	2.50	3.01	1.20
Bmag6	7	0.608	2.55	4.26	1.35
EBmac0701	6	0.765	4.26	4.59	1.60
Bmac310	5	0.713	3.48	3.57	1.37
Bmag0223	4	0.643	2.80	2.57	1.10
EBmac602	4	0.557	2.26	2.23	1.02
Bmag007	8	0.812	5.31	6.49	1.84

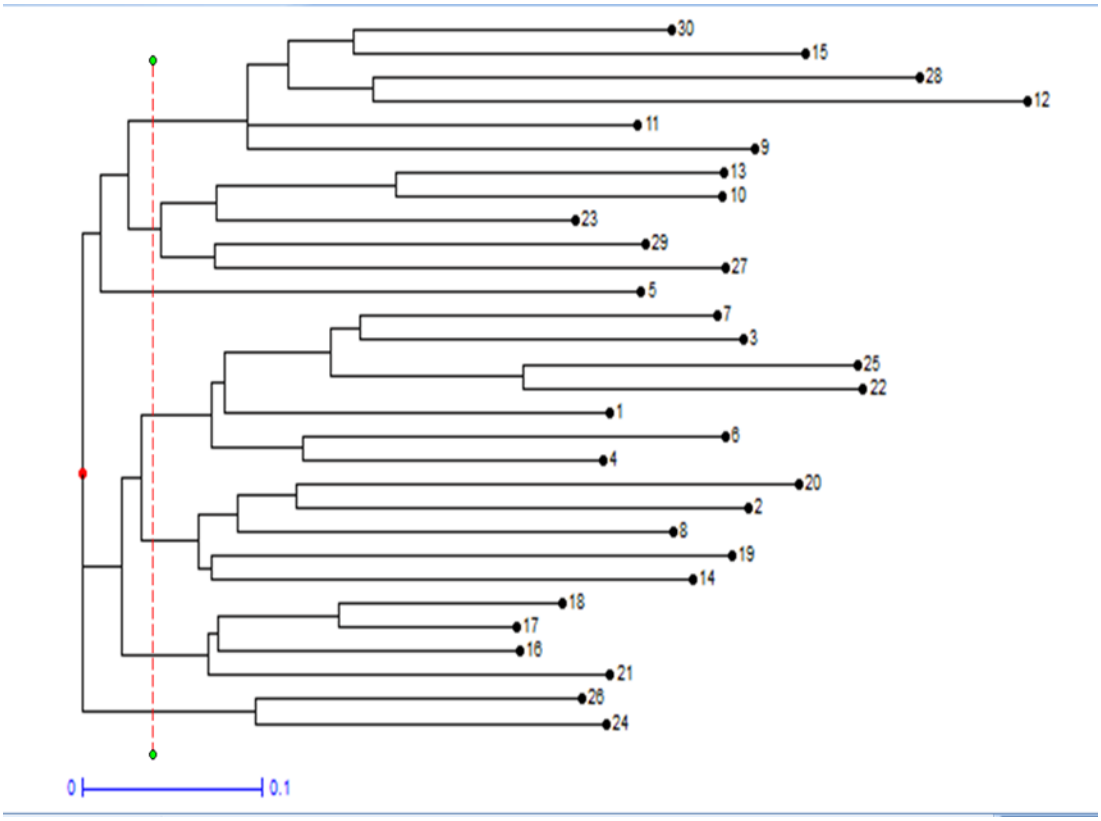

Figure 1. A dendrogram based on SSR markers of the 30 *H. spontaneum* populations by UPGMA method.

The first cluster consisted of six genotypes of Iranian accession (Kermanshah NO. 30), Iranian accession (Kurdistan), IG38936 (Palestine), IG137596 (Armenia), IG144170 (Jordan), and IG39565 (Palestine). The second cluster included five accessions such as IG 120793 (Turkmenistan), IG 140189 (Tajikistan), IG40152 (Uzbekistan), Iranian accession (Kermanshah NO. 29), and IG140302 (Tajikistan). The third cluster had only one accession, IG117888 (Syria). The fourth cluster comprised seven accessions, IG 39919 (Syria), IG38669 (Afghanistan), IG 39489 (Syria), IG119438 (Syria), IG120790 (Turkmenistan), IG120794 (Turkmenistan), and IG40155 (Uzbekistan). The fifth cluster included five accessions, IG139141 (Jordan), IG38654 (Afghanistan), IG120794 (Turkmenistan), IG121853 (Syria), and IG129152 (Iraq). The sixth cluster had four accessions, including IG38657 (Afghanistan), IG119447 (Syria), IG142356 (Tajikistan), and IG142486 (Tajikistan). The seventh cluster included two accessions, (IG 140073 (Tajikistan) and IG140352 (Tajikistan).

Population structure

K and ΔK statistics were extracted by determining the population structure using the MCMC algorithm. The maximum value was K=7 (Figure 2). Thus, the 30 *H. spontaneum* accessions were divided into seven subpopulations (Figure 2). All subpopulations had the same proportion of membership (0.143). Therefore, the same number of accessions was observed in each subpopulation. The highest genetic differentiation belonged to the fourth subpopulation (0.0743). The seventh subpopulation had the lowest genetic differentiation (0.0002) (Table 6).

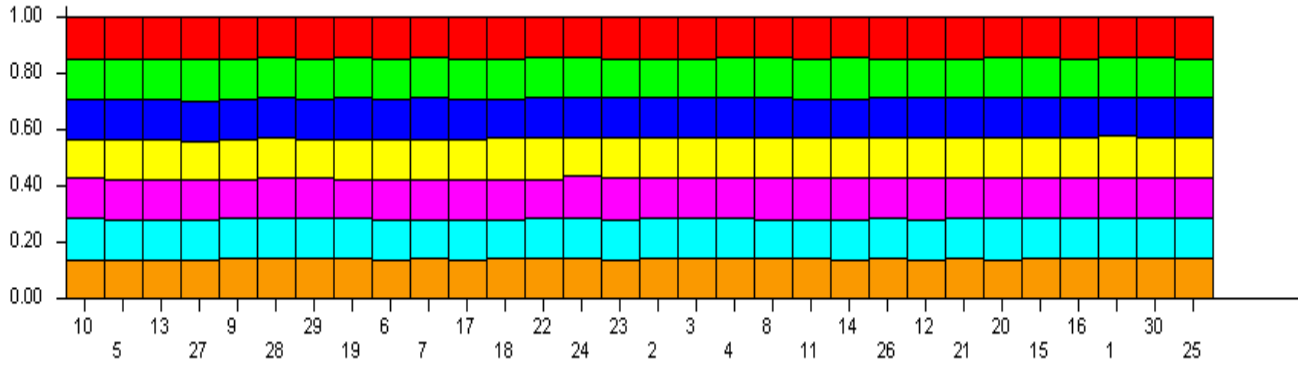

Figure 2. Genetic relatedness of 30 *H. spontaneum* populations with 10 SSR primer combinations as analyzed by the STRUCTURE program.

Table 6. Percentage of accessions, differentiation index and genetic distance of the extracted subpopulations.

Subpopulation	Proportion of membership	differentiation index (fst)	Genetic distance						
			1	2	3	4	5	6	7
1	0.143	0.0363	-	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
2	0.143	0.0050	0.0000	-	0.0000	0.0001	0.0000	0.0000	0.0000
3	0.143	0.0386	0.0000	0.0000	-	0.0000	0.0000	0.0000	0.0000
4	0.143	0.0743	0.0000	0.0001		-	0.0000	0.0001	0.0001
5	0.143	0.0007	0.0000	0.0000	0.0000	0.0001	-	0.0000	0.0000
6	0.143	0.0183	0.0000	0.0000	0.0000	0.0001		-	0.0000
7	0.143	0.0002	0.0000	0.0000	0.0000	0.0001	0.0000		-

Association analysis

Association analysis was conducted with only 11 marker loci, as they exhibited a minor allele frequency (i.e., below 5%) (Table 7). Bmag007 (on 7H), Bmag6 (on the 3H), and Bmac0399 (on the 1H) were significantly associated with leaf rolling in two loci. Bmag0125 (on the 2H) was significantly associated with Chlorophyll a, Total Chlorophyll, and Chlorophyll b. Furthermore, there were significant associations between the marker EBmac0701 (on the 4H) and Total Chlorophyll, Chlorophyll b, Chlorophyll a, and chlorosis. Further, EBmac0701 (on the 4H) was significantly associated with leaf chlorosis (Table 7).

Discussion

This study contributes new insights into the genetic diversity among *H. spontaneum* populations for RWA-resistance, offering valuable information for integration into the breeding programs aimed at improving RWA resistance in barley cultivars. IG120794 collected from Turkmenistan showed the least leaf chlorosis and rolling, which reveal its potential for using in a barley-breeding program in future. High resistance in recent study could be considered as a valuable *D. noxia* resistance source that could be exploited by development of resistant *H. vulgare* germplasms through a backcross program. Although they lacked the 2HS resistance QTL, DH lines with a moderate resistance impact were reported to introgress aphid resistance from the wild barley source *H. spont* (Ahman and Bengtsson, 2019). Leybourne et al. (Kulwal and Singh, 2021) showed that *H. spont*. possesses partial resistance involved elevated basal expression of thionin and phytohormone signaling genes as well as a reduction in phloem quality.

The effect of feeding by this aphid species can be assessed by both chlorophyll and carotenoid concentrations. IG129152 genotype had the lowest chlorophyll a, chlorophyll b, and total chlorophyll concentration, and was the most susceptible population. Chlorosis is the loss of chlorophylls (i.e., chlorophylls a and b) caused by *D. noxia* feeding. Prior studies have thoroughly investigated *D. noxia*-induced chlorophyll reductions and their implications on plant photosynthetic efficiency (Fouche et al., 1984; Burd and Elliott, 1996; Gupta et al., 2005; Macaulay et al., 2020). Likewise Burd and Elliott (1996) compared the chlorophyll content kinetics in susceptible and resistant plants and found that *D. noxia* feeding reduced chlorophyll a, b, and total content in susceptible wheat (Tavon' and 'TAM W-IOl') and barley ('Wintermalt') but not in resistant plants. Resistant plants seem to counteract the deleterious effects of aphid herbivory on leaves through up-regulation of detoxication mechanisms and faster regeneration of photosynthetic active centers and RuBP.

Table 7. Marker-trait associations using MLM and GLM models.

Traits	Marker name	No. of associations	P. value		R^2(%)
			MLM	GLM	
Leaf rolling	Bmag007	2	0.0056	0.0056	0.2455
Chlorophyll a	Bmag0125	3	0.0107	0.0093	0.1975
Total Chlorophyll	Bmag0125	3	0.0112	0.0096	0.1919
Chlorophyll b	Bmag0125	3	0.0127	0.0109	0.1841
Leaf rolling	Bmag007	2	0.0149	0.0149	0.1976
Total Chlorophyll	EBmac0701	3	0.0289	0.0289	0.1428
Chlorophyll b	EBmac0701	3	0.0296	0.0296	0.1399
Chlorophyll a	EBmac0701	3	0.0306	0.0306	0.1433
Leaf rolling	Bmac0399	1	0.0342	0.0342	0.1545
Leaf chlorosis	EBmac0701	4	0.0344	0.0344	0.1369
Leaf rolling	Bmag6	1	0.045	0.045	0.1400

In contrast, leaves of susceptible plants are unable to sustain these processes and become senescent (Franzen et al., 2014).

Gray et al. (1990) determined the association between marker alleles and phenotypes in the homogeneous subpopulations by focusing on the fundamental idea of a population-based mixed population into several unstructured subpopulations. At K = 7, the 30 *H. spontaneum* populations applied for association analysis were divided into seven subpopulations in this study. Subpopulations within a population may exist owing to differences in genotype, geographical origin, natural or human selection, or genetic drift 8). Bayesian clustering in STRUCTURE, PCoA and AMOVA analyses revealed that the population might be differentiated mainly due to the different breeding program origins and by ear row type and divided into five subpopulations (Capo-Chichi et al., 2022). Several reports show that both geographical origin and ear-row type are factors influencing barley population structure (Bengtsson et al., 2017; Capo-Chichi et al., 2022). The geographical separation of the barley genotypes observed in this study could also be justified by local adaptation associating with alterations in geographical attributes in the regions of origin. Genetic diversity, manifested through allelic variants of the involved genes, is a critical factor in selecting the candidate parents to improve a desired agronomic trait.

Association mapping enabled a much larger pool of germplasm to be analyzed for regions related to RWA resistance than the method used in the alternative approaches which depend on biparental mapping populations. SSR markers Bmag007, Bmag6, Bmac0399, Bmag0125, and EBmac0701 exhibited significant associations with RWA resistance traits. As expected, the considerably expanded pool of *H. spontaneum* populations shed light on the genetics of RWA resistance and identified new regions associated with resistance including loci on 1H, 2H, 3H, 4H, and 7H chromosomes. Previous studies identified loci on lH and (Nei, 1973; Macedo, 2003; Mittal et al., 2008; Radchenko et al., 2022). Resistance to RWA in barley, analyzed through QTL analysis and genome-wide association mapping, showed multiple relevant genes, including sites on chromosomes 2H and 3H (Nei, 1973; Dahleen et al., 2012; Leybourne et al., 2019; Singh et al., 2023).

Conclusion

the genetic diversity within wild barley accessions presents a valuable resource for enhancing the genetic foundation of barley and developing superior cultivars. Employing association analysis with a diverse array of wild barley accessions proves effective in pinpointing Marker-Trait Associations (MTAs) for traits such as RWA resistance. The significant markers identified in this study, once validated, can be integrated into marker-assisted breeding programs. Moreover, the preliminary association analysis results for RWA resistance provide a foundation for screening genotypes with aphid tolerance. Future exploration of the putatively linked genomic regions holds promise for uncovering crucial insights into RWA resistance mechanisms in barley.

Supplementary Materials

No supplementary material is available for this article.

Author Contributions

Supervision of the study, Z.T.; Collection of experimental data and writing the manuscript, S.S.; molecular and statistical analysis, A.A.; review of the manuscript, F.F.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

References

Able, J.A., Langridge, P., and Milligan, A.S. (2007). Capturing diversity in the cereals: many options but little promiscuity. *Trends Plant Sci* 12(2): 71-79. doi: 10.1016/j.tplants.2006.12.002.

Ahman, I., and Bengtsson, T. (2019). Introgression of resistance to *Rhopalosiphum padi* L. from wild barley into cultivated barley facilitated by doubled haploid and molecular marker techniques. *Theor Appl Genet* 132: 1397-1408.

Alvarez, M.F., Mosquera, T., and Blair, M.W. (2014). The use of association genetics approaches in plant breeding. *Plant Breed Rev* 38: 17-68.

Arora, S., Steuernagel, B., Gaurav, K., Chandramohan, S., Long, Y., Matny, O., Johnson, R., Enk, J., Periyannan, S., Singh, N., Asyraf Md Hatta, M., Athiyannan, N., Cheema, J., Yu, G., Kangara, N., Ghosh, S., Szabo, L.J., Poland, J., Bariana, H., et al. (2019). Resistance gene cloning from a wild crop relative by sequence capture and association genetics. *Nat Biotechnol* 37(2): 139-143. doi: 10.1038/s41587-018-0007-9.

Bengtsson, T., Manninen, O., Jahoor, A., and Orabi, J. (2017). Genetic diversity, population structure and linkage disequilibrium in Nordic spring barley (*Hordeum vulgare* L. subsp. vulgare). *Genet Resour Crop Evol* 64: 2021-2033.

Bradbury, P.J., Zhang, Z., Kroon, D.E., Casstevens, T.M., Ramdoss, Y., and Buckler, E.S. (2007). TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics* 23(19): 2633-2635. doi: 10.1093/bioinformatics/btm308.

Burd, J.D., and Elliott, N.C. (1996). Changes in chlorophyll a fluorescence induction kinetics in cereals infested with Russian wheat aphid (*Homopetra: Aphididea*). *J Econ Entomol* 89(5): 1332-1337.

Cakir, M., Vitou, J., Kollehn, D., Lawson, W., Ilbi, H., Haley, S., Peairs, F., Mornhinweg, D., Bohssini, M., and Ogbonnaya, F. (2009). "A global effort in breeding for resistance to Russian wheat aphid in wheat and barley", in: *19th International Triticeae Mapping Initiative - 3rd COST Tritigen.* (Clermont-Ferrand, France).

Capo-Chichi, L.J.A., Elakhdar, A., Kubo, T., Nyachiro, J., Juskiw, P., Capettini, F., Slaski, J.J., Ramirez, G.H., and Beattie, A.D. (2022). Genetic diversity and population structure assessment of Western Canadian barley cooperative trials. *Front Plant Sci* 13: 1006719. doi: 10.3389/fpls.2022.1006719.

Collard, B.C., and Mackill, D.J. (2008). Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philos Trans R Soc Lond B Biol Sci* 363(1491): 557-572. doi: 10.1098/rstb.2007.2170.

Dahleen, L.S., Bregitzer, P., Mornhinweg, D., and Jackson, E.W. (2012). Association mapping of Russian wheat aphid resistance in barley as a method to identify diversity in the national small grains collection. *Crop Sci* 52(4): 1651-1662.

Dahleen, L.S., Bregitzer, P., Mornhinweg, D., and Klos, K.E. (2015). Genetic diversity for Russian wheat aphid resistance as determined by genome‐wide association mapping and inheritance in progeny. *Crop Sci* 55(5): 1925-1933.

Fouche, A., Verhoeven, R., Hewitt, P., Walters, M., Friel, C., and De Jager, J. (1984). Russian aphid (*Diuraphis noxia*) feeding damage on wheat, related cereals and a *Bromus* grass species. *Tech commun - Repub S Afr Dep Agric*: 22–33.

Franzen, L.D., Gutsche, A.R., Heng-Moss, T.M., Higley, L.G., Sarath, G., and Burd, J.D. (2014). Physiological and biochemical responses of resistant and susceptible wheat to injury by Russian wheat aphid. *J Econ Entomol* 100(5): 1692-1703.

Gharaghanipor, N., Arzani, A., Rahimmalek, M., and Ravash, R. (2022). Physiological and transcriptome indicators of salt tolerance in wild and cultivated barley. *Front Plant Sci* 13: 819282. doi: 10.3389/fpls.2022.819282.

Girma, M., Wilde, G.E., and Harvey, T. (1993). Russian wheat aphid (*Homoptera*: *Aphididae*) affects yield and quality of wheat. *J Econ Entomol* 86(2): 594-601.

Gray, S.N., Robinson, P., Wilding, N., and Markham, P. (1990). Effect of oleic acid on vegetative growth of the aphid-pathogenic fungus *Erynia neoaphidis*. *FEMS Microbiol Ecol* 68(1-2): 131-136.

Gupta, P.K., Rustgi, S., and Kulwal, P.L. (2005). Linkage disequilibrium and association studies in higher plants: present status and future prospects. *Plant Mol Biol* 57(4): 461-485. doi: 10.1007/s11103-005-0257-z.

Heng-Moss, T., Ni, X., Macedo, T., Markwell, J.P., Baxendale, F.P., Quisenberry, S., and Tolmay, V. (2003). Comparison of chlorophyll and carotenoid concentrations among Russian wheat aphid (*Homoptera*: *Aphididae*)-infested wheat isolines. *J Econ Entomol* 96(2): 475-481.

Joukhadar, R., El-Bouhssini, M., Jighly, A., and Ogbonnaya, F. (2013). Genome-wide association mapping for five major pest resistances in wheat. *Mol Breed* 32: 943-960.

Kisten, L., Tolmay, V.L., Mathew, I., Sydenham, S.L., and Venter, E. (2020). Genome-wide association analysis of Russian wheat aphid (*Diuraphis noxia*) resistance in Dn4 derived wheat lines evaluated in South Africa. *PLoS One* 15(12): e0244455. doi: 10.1371/journal.pone.0244455.

Kulwal, P.L., and Singh, R. (2021). Association mapping in plants. *Methods Mol Biol* 2264: 105-117. doi: 10.1007/978-1-0716-1201-9_8.

Leybourne, D.J., Valentine, T.A., Robertson, J.A.H., Perez-Fernandez, E., Main, A.M., Karley, A.J., and Bos, J.I.B. (2019). Defence gene expression and phloem quality contribute to mesophyll and phloem resistance to aphids in wild barley. *J Exp Bot* 70(15): 4011-4026. doi: 10.1093/jxb/erz163.

Macaulay, M., Ramsay, L., and Åhman, I. (2020). Quantitative trait locus for resistance to the aphid *Rhopalosiphum padi* L. in barley (*Hordeum vulgare* L.) is not linked with a genomic region for gramine concentration. *Arthropod-Plant Int* 14: 57-65.

Macedo, T.B. (2003). *Physiological responses of plants to piercing-sucking arthropods.* Doctora of Philosophy, The University of Nebraska-Lincoln.

Mittal, S., Dahleen, L.S., and Mornhinweg, D. (2008). Locations of quantitative trait loci conferring Russian wheat aphid resistance in barley germplasm STARS - 9301B. *Crop Sci* 48(4): 1452-1458.

Moreira, X., Abdala-Roberts, L., Gols, R., and Francisco, M. (2018). Plant domestication decreases both constitutive and induced chemical defences by direct selection against defensive traits. *Sci Rep* 8(1): 12678.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proc Natl Acad Sci U S A* 70(12): 3321-3323. doi: 10.1073/pnas.70.12.3321.

Nieto Lopez, R.M., and Blake, T.K. (1994). Russian wheat aphid resistance in barley: inheritance and linked molecular markers. *Crop Sci* 34(3): 655-659.

Oliver, R.E., Obert, D.E., Hu, G., Bonman, J.M., O'Leary-Jepsen, E., and Jackson, E.W. (2010). Development of oat-based markers from barley and wheat microsatellites. *Genome* 53(6): 458-471. doi: 10.1139/g10-021.

Pritchard, J.K., Stephens, M., and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* 155(2): 945-959. doi: 10.1093/genetics/155.2.945.

Radchenko, E.E., Abdullaev, R.A., and Anisimova, I.N. (2022). Genetic resources of cereal crops for Aphid resistance. *Plants* 11(11): 1490. doi: 10.3390/plants11111490.

Raman, H., and Read, B.J. (2000). Molecular breeding for resistance against Russian wheat aphid in Australian barley. *J Agric Genome* 5: 1-7.

Rohlf, F.J. (1988). *NTSYS-pc: numerical taxonomy and multivariate analysis system.* Exeter Publishing.

Singh, B., Bhatia, D., Narang, D., Kaur, R., and Chhuneja, P. (2023). High-resolution genetic mapping of QTL governing resistance to corn leaf aphid, *Rhopalosiphum maidis* (Fitch) in barley. *Cereal Res Commun* 51(2): 379-389.

Tocho, E., Ricci, M., Tacaliti, M., Giménez, D., Acevedo, A., Lohwasser, U., Börner, A., and Castro, A. (2012). Mapping resistance genes conferring tolerance to RWA (*Diuraphis noxia*) in barley (*Hordeum vulgare*). *Euphytica* 188: 239-251.

Tolmay, V., Jankielsohn, A., and Sydenham, S. (2013). Resistance evaluation of wheat germplasm containing *Dn4* or *Dny* against R ussian wheat aphid biotype RWASA 3. *J Appl Entomol* 137(6): 476-480.

Tolmay, V.L., Sydenham, S.L., Sikhakhane, T.N., Nhlapho, B.N., and Tsilo, T.J. (2020). Elusive diagnostic markers for Russian wheat aphid resistance in bread wheat: deliberating and reviewing the status quo. *Int J Mol Sci* 21(21): 8271. doi: 10.3390/ijms21218271.

Webster, J., Baker, C., and Porter, D. (1991). Detection and mechanisms of Russian wheat aphid (*Homoptera*: *Aphididae*) resistance in barley. *J Econ Entomol* 84(2): 669-673.

Zhang, H., Mittal, N., Leamy, L.J., Barazani, O., and Song, B.H. (2017). Back into the wild—Apply untapped genetic diversity of wild relatives for crop improvement. *Evol Appl* 10(1): 5-24.

Zohary, D., and Hopf, M. (2000). *Domestication of plants in the Old World: The origin and spread of cultivated plants in West Asia, Europe and the Nile Valley*. Oxford University Press.

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مقاله پژوهشی

ارتباط نشانگر صفت مقاومت شته گندم روسی (*Diuraphis noxia*) در مجموعه متنوع جهانی جو وحشی

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چکیده: شته روسی گندم (*Diuraphis noxia* Kurdjumov ،RWA) به عملکرد و کیفیت دانه جو به شدت آسیب می‌رساند. مطالعه حاضر با هدف شناسایی نشانگرهای ردیف‌های تکراری ساده (SSR) مرتبط با مقاومت به RWA در جو وحشی (*Hordeum vulgare* subsp. *spontaneum*) انجام شد. جمعیتی متشکل از ۳۰ نمونه جو وحشی، از بانک ژن ایکاردا و ایران که از ۱۰ کشور آسیایی در امتداد پراکندگی جغرافیایی طبیعی گونه بودند، انتخاب شد. برای مطالعه تنوع ژنتیکی از ۱۴ نشانگر SSR استفاده شد. ارتباط ۵۲ نشانگر SSR چندشکلی با صفات مقاومت RWA مرتبط با استفاده از روش‌های مدل خطی عمومی (GLM) و مدل خطی مختلط (MLM) مورد آزمایش قرار گرفت. تجزیه و تحلیل ساختار جمعیت هفت زیرجمعیت را در کل مجموعه نشان داد (K=7). یازده نشانگر مرتبط با صفت روی کروموزوم‌های ۱H، ۲H، ۳H، ۴H و ۷H شناسایی شد. نشانگرهای Bmag007، Bmag6، Bmac0399، Bmag0125 و EBmac0701 ارتباط معنی‌داری با صفات مقاومت به RWA نشان دادند. هر پنج نشانگر در هر دو روش GLM و MLM با صفات مرتبط بودند. این مطالعه مناطق ژنومی جدیدی را شناسایی نمود که با صفات مقاومت به RWA مرتبط هستند، که می‌تواند برای درک معماری ژنتیکی مقاومت به RWA در جو مورد مطالعه قرار گیرد.

کلمات کلیدی: تجزیه ارتباطی، زیرجمعیت، مقاومت گیاهچه‌ای، نشانگر SSR .

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