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Identification of genetic resources of field resistance to barley leaf rust in local germplasm of cultivated barley

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Abstract

The barley leaf rust has been important in recent years in Iran. In order to identify the genetic resources of resistance to this disease, 207 Iranian barley landraces were studied. The germplasms were investigated at the field of Iraqi-Mahalleh research station in Gorgan as the disease hotspot under natural incidence over three years. The results showed that four genotypes including KC18638 and KC18643 from Golestan and KC19087 and KC 19093 from Gilan expressed immunity response in all three years of the experiment and 31 accessions were immune in two years. The results of principal component analysis in three years showed that 90.69% of the variations in the data were justified by the first three principal components. The results of this study indicated the possibility of racial variation in the studied disease hotspot. These findings show the importance of tracing the changes in the disease hotspot which is necessary for planning, breeding and cultivation of cultivars with effective resistance. Also, the resistant genotypes identified in this research can be used as genetic resources of leaf rust resistance in breeding programs.

Key words: Barley brown rust, Diversity, Landrace, Resistance components.

INTRODUCTION

Barley is an important crop for livestock feeding, malt production, and human consumption and is ranked fourth in terms of global production after corn, rice and wheat. Global production of barley was 133 million metric tons from 50 million hectares in 2012 (FAOSTAT, 2015). Barley is known as one of the foundations of modern agriculture. This crop was domesticated about 10,000 years ago from its ancestor (*Hordeum vulgare* spp. *spontaneum*), in the Fertile Crescent (Badr *et al.,* 2000). Barley is a hard and resistant cereal that grows in a range of environments difficult for other crops to grow in terms of altitude or latitude.

Leaf rust of barley is a fungal disease caused by *Puccinia hordei*. The importance of barley leaf rust has increased in recent decades in regions with temperate weather conditions, which is probably due to intensive farming operations (Park *et al.,* 2015). However, the economic importance of this disease depends on its prevalence in the world and varies from year to year (Niks *et al.,* 2000). Barley leaf rust is the most prevalent in barley cultivation areas of North Africa, Europe, New Zealand, Australia, the eastern and middle western parts of the United States, and parts of Asia, which causes severe yield loss in susceptible cultivars (Park *et al.,* 2015). The presence of this disease in Iran was first reported from Mazandaran by Sharif and Ershad (Ershad, 1995). This disease has been distributed sporadically in some parts of the country, including Azerbaijan, Khorasan, Khuzestan, Mazandaran and Ilam (Bamdadian and Torabi, 1983). However, in recent years, this disease has spread a lot in many areas of barley cultivation of Iran, especially in the northern

provinces of the country, so that it appeared as epidemic in the spring of 2010 in these provinces (Abdolkarim *et al.,* 2011). To date, 21 loci of seedling resistance (including *Rph1* to *Rph19*, *Rph21* and *Rph22*) and two adult plant resistance genes (*Rph20* and *Rph23*) have been identified for barley leaf rust in *H. vulgare*, *H. vulgare* subsp. *spontaneum* and *H. bulbosum* (Park, 2003, Hickey *et al.,* 2011, Sandhu *et al.,* 2012). Various investigations have been conducted to identify the genetic resources of the resistance to barley leaf rust. Jin *et al.* (1995) assessed 1997 accessions of *H. vulgare* and 885 accessions of *H. spontaneum*, which originated mostly from the Mediterranean and parts of North Africa, with the isolate ND89-3 at the seedling stage to identify new sources of resistance to *P. hordei*. The results indicated that 58 accessions of *H. vulgare* and 222 accessions of *H. spontaneum* had a lower infection type to this isolate. They stated that exploitation of these accessions would widen the available germplasm resources for genetic control of *P. hordei*. Results of filed evaluation to investigate the resistance level to *P. hordei* in modern spring barley germplasm by Niks *et al.* (2000) showed that most of the genotypes were significantly less infected than Grit, a moderate-resistant check cultivar, in some areas and years. They concluded that higher levels of relative resistance to *P. hordei* in spring barley germplasm have been attained through breeding attempts. Shtaya *et al.* (2006) screened 418 Spanish accessions of barley for leaf rust resistance in field condition in Córdoba, Spain, and identified six accessions with the least disease severity and no symptoms of visible macroscopic necrosis. Steffenson *et al.* (2007) evaluated 318 accessions of *H. vulgare* ssp. *spontaneum* belonging to Wild Barley Diversity Collection originating from the Fertile Crescent, Central Asia, North Africa and the Caucasus for resistance to leaf rust. The accessions showed a wide range of infection type and the frequency of resistance was 25.8%. The results of this study showed that the wild species *H. vulgare* ssp. *spontaneum*is a rich source for resistance to leaf rust. Silvar *et al.* (2010) evaluated the Spanish core collection consisting of 159 inbred lines derived from indigenous genotypes and 16 cultivars adapted to southern European conditions for resistance to leaf rust. The results indicated that only a small number of genotypes showed resistance levels as well as controls. In other words, the frequency of rust resistance in this germplasm collection was low. Furthermore, inbred lines derived from indigenous genotypes of the Mediterranean coast and southern Europe were the most resistant genotypes.

Limited studies have been conducted on the resistance to *P. hordei* in Iran. Dehghan and Pour-Mansouri (2010) evaluated resistance reaction of 70 landraces of barley collection of National Genetic Bank Iran for leaf rust at Golestan Agriculture Research Station in field condition. The results of the experiment showed that 24% of the studied populations possessed favorable levels of resistance to the disease. Abdolkarim *et al.* (2011) determined the virulence factors and pathotypes of *P. hordei* by collecting infected barley plants from different regions of the country by using isogenic lines based on virulence-avirulnece formulas. Considering the importance of this disease, its spread in the country in recent years and the limited research carried out on the identification of sources of resistance, this study aimed to investigate the local barley germplasm in order to identify the resistance sources to barley leaf rust.

MATERIALS AND METHODS

This research was conducted in three successive growing years of 2015-2016, 2016-2017 and 2017-2018 in the field of the Iraqi-Mahalleh research station of Gorgan as the disease hotspot of barley leaf rust. A total of 207 local accessions from barley collection of National Plant Gene Bank of Iran were cultivated in rows of 1 meter length and 30 cm spacing between rows. For each of the ten rows, a row of Afzal cultivar was cultivated as the spreader of the disease. Resistance evaluation was carried out when the flag leaf of the susceptible cultivar Afzal was 100% infected. For this purpose, two types of resistance components, including infection type and disease severity were measured. Evaluation of the disease severity was performed based on the modified Cobb's Scale (Peterson *et al.,* 1948) and the evaluation of the infection type was conducted based on O (immune), R (resistant), MR (Moderately-resistant), M (Intermediate), MS (Moderately-susceptible) and S (susceptible) scale according to Roelfs method (Roelfs *et al.,* 1992). The coefficient of infection was then calculated by multiplying the values of disease severity by the values of the infection type, taking into account the values of 0, 0.2, 0.4, 0.6, 0.8 and 1 for groups O, R, MR, M, MS And S, respectively. Descriptive statistics for the resulting quantitative measurement (disease severity) were investigated. The correlation of coefficient between the resistance components was calculated for each year and between different years. Relatedness of variables in correlation matrix was tested by Bartlett's test of sphericity. The conformity of the resistance reaction of the studied accessions through different years of the assessment was also investigated by fitting regression

lines on the data measured in different combinations of two years. Genetic distances among the genotypes were calculated based on resistance components. The agreement of resulting matrices of genetic distances was investigated by Mantel test (Mantel, 1967) through comparison with a reference distribution developed by Monte-Carlo approach. In order to reduce the amount of data and extracting distinctive indices, the principal component analysis was performed based on resistance traits. Differentiation of the accessions based on their origin was also studied. For this purpose, the technique of Discriminant Analysis of Principal Components (DAPC), introduced by Jombart *et al.* (Jombart *et al.,* 2010) was used. Cluster analysis was performed with K means method by calculating Bayesian Criterion (BIC) for choosing K (number of members in each group) optimally. Statistical analysis and plotting of graphs was performed by coding and using adegenet package in the R software, version 3.5.1.

RESULTS

By comparing infection type of the accessions studied in the three years of the experiment, a total of 21 different reaction patterns were identified (Table 1). In group 1, four accessions including KC 18638 and KC 18643 from Golestan and KC 19087 and KC 19093 from Gilan showed immune response in all years. The second to fourth groups consisted of accessions that showed at least two years of immunity response. Eleven accessions were included in the second group consisting of KC 19104 and KC 19108 from Gilan, KC 18639, KC 18640 and KC 18646 from Golestan and KC 18530, KC 18534, KC 20252, KC 20253, KC 20254 and KC 20255 from Mazandaran showing moderate-resistance in the first year and immunity in the second and third years. Twelve accessions including KC 18652 from West Azerbaijan, KC 20557, KC 20558, KC 20559 and KC 20560 from East Azarbaijan, KC 19088 and KC 19107 from Gilan, KC 18419 and KC 19925 from Golestan and KC 18532, KC 19940 and KC 20231 from Mazandaran were placed in the third group which showed moderate-susceptibility in the first year and immunity in the second and third years of experiment. Eight accessions were located in the fourth group. These accessions included KC 19096 and KC 19105 from Gilan, KC 20248 from Golestan, and KC 19943, KC 19959, KC 20057, KC 20230 and KC 20262 from Mazandaran, which showed susceptibility in the first year and immunity in the second and third years. The accessions in groups 5 to 16 (68 accessions, totally) exhibited resistance or immunity response in one year of experiment. Groups five to eight (including

Table 1. Patterns of infection type and mean severity in filed response of local barley landraces to leaf rust.

Group number	Reaction pattern	Number of members	Mean severity		
			2015-2016	2016-2017	2017-2018
1	0-0-0	4	0	0	0
2	MR-0-0	11	13.18	0	0
3	MS-0-0	12	28.00	0	0
4	S-0-0	8	51.25	0	0
5	0/R-S-S/MR	2	1.67	53.33	63.33
6	MR-0-MS	5	17.00	0	34.00
7	MR-0-S	5	10.00	0	62.00
8	MR-S-0	5	17.00	22.00	0
9	MS-0-MS	8	27.50	0	32.50
10	MS-0-S	7	31.43	0	84.29
11	S-0-MS	2	55.00	0	35.00
12	MS-S-0	5	28.00	36.00	0
13	S-S-0	8	53.75	53.75	0
14	S-0-S	12	57.50	0	85.83
15	0-S-S	4	0	50.00	60.00
16	MR-S-MS	5	15.00	44.00	38.00
17	MR-S-S	10	15.00	36.00	79.00
18	MS-S-MS	5	20.00	44.00	24.00
19	MS-S-S	21	26.19	44.29	77.62
20	S-S-MS	8	56.25	40.00	35.00
21	S-S-S	60	61.83	52.92	91.83

17 accessions) expressed moderate-resistance response in one of three years of experiment. Groups 17, 19 and 20 in two years and group 21 in all three years of experiment showed susceptibility.

Comparison of the results of three years of experiment showed that the mean of coefficient of infection was higher in 2017-2018 than those of the other two years (Table 2). A significant and strong correlation was found between infection type and disease severity in all three years (Table 3). Correlation between the estimates of coefficient of infection in different years was significant (despite the fact that it was not high). In the regression analysis for coefficient of infection estimated in different years in a two to two manner, the largest slope of the line (b=0.63) belonged to the regressions of 2016-2017 and 2017-2018 (Table 4).

The χ^2 value in sphericity test was highly significant indicating that the variables in correlation matrix are related and suitable enough for structure detection by principal components analysis. The results of principal component analysis showed that 90.69% of the variation in the data was justified by the three principal components, each explaining 51.92%, 21.49% and 17.28% of the total variance, respectively (Table 5). In the first component, the sign of the coefficients of all traits was positive, therefore, the lower values of this component indicates lower disease severity and infection type in all years of the experiment. The accessions KC 18638, KC 18643, KC 19087 and KC 19093 showed the lowest values for the first principal

Table 2. Descriptive statistics of coefficient of infection in filed response of local barley landraces to leaf rust.

Year	Minimum	Maximum	Range	Mean	Standard deviation	Coefficient of variation (%)
2015-2016	0.00	0.90	0.90	0.35	0.27	77.87
2016-2017	0.00	1.00	1.00	0.30	0.28	90.70
2017-2018	0.00	1.00	1.00	0.58	0.40	69.95

Table 3. Coefficients of correlation between infection type and disease severity (a) and between estimates of coefficient of infection (b) of local barley accessions against barley leaf rust during three years of evaluation in Gorgan hotspot of the disease.

Year	2015-2016	2016-2017	2017-2018
(a)	0.747**	0.834**	0.864**
(b)			
2015-2016		0.376**	0.401**
2016-2017		0.422**	

**: Significant at the probability level of 0.01

Table 4. The results of regression for coefficient of infection against barley leaf rust in local barley accessions during three years of evaluation in Gorgan hotspot of the disease.

Regression	Estimate	Standard deviation	t
(2016-2017)/(2015-2016)			
Intercept	0.17	0.03	5.89***
Coefficient	0.37	0.07	5.64***
(2017-2018)/(2015-2016)			
Intercept	0.32	0.04	7.54***
Coefficient	0.61	0.10	6.28***
(2017-2018)/(2016-2017)			
Intercept	0.34	0.04	5.89***
Coefficient	0.63	0.09	5.64***

***: Significant at the probability level of 0.001

component. In the second principal component, coefficients of disease severity and the infection type were positive for 2015-2016 and negative for 2016-2017 and 2017-2018. Therefore, the higher values of this principal component distinguishes the accessions with lower disease severity and infection type in 2016-2017 and 2017-2018 and higher disease severity and infection type in 2015-2016. The accessions KC 19096, KC 19943, KC 20057 and KC 20230 had the highest values for the second principal component. Also, lower values for this principal component, discriminates the accessions with higher disease severity and the infection type in 2016-2017 and 2017-2018 and lower disease severity and infection type in 2015-2016. The accessions KC 18649, KC 19121, KC 18636, KC 18644, KC 18536, KC 18032 and KC 18605 had the least values for the second principal component. In the third principal component, coefficients of disease severity and the infection type were positive for 2015-2016 and 2016-2017 and were negative for 2017-2018. Therefore, the higher values of this component differentiate the accessions with higher disease severity and infection type in 2015-2016 and 2016-2017. The accessions KC 20981, KC 19958, KC 20974, KC 20196, KC 20259, KC 19957, KC 20555 and KC 20271 had the highest values of the third principal component. Also, lower values of the third principal component distinguishes the accessions with lower disease severity and infection type in 2015-2016 and 2016-2017 and higher disease severity and infection type in 2017-2018. Accessions KC 18415, KC 19931 and KC 20249 had the lowest values of the third principal component.

In the biplot of the first and second principal components (Figure 1), the accessions that generally showed low infection type and disease severity during the three years of the experiment were located in the left half of the biplot based on the characteristics of the first component (PC1), which includes the upper left (second quarter) and lower left (third quarter). Based on the characteristics of the second principal component (PC2), the accessions in the second quarter appeared to be more resistant in the years 2016-2017 and 2017-2018, while accessions in the third quarter showed more resistance in 2015-2016. The second and third quarters consisted of 53 and 45 accessions, respectively.

Based on values of Wricke equivalence (wi^2), accessions KC 20974, KC 19958 and KC 20981 had the highest changes in the coefficient of infection during the three years of the experiment (Table 6). Twelve genotypes including KC 18034, KC 18035, KC 19090, KC 18426, KC 19112, KC 20191, KC 20229, KC 20192, KC 20193, KC 20195, KC 20267 and KC 20458 lied more than one standard deviation from total mean of wi^2 which indicates the highest stability in the coefficient of infection during the period of the experiment. Among these accessions, KC 18034, KC 18035, KC 19090, KC 18426 and KC 19112 expressed mainly moderate-resistance or moderate-susceptibility and KC 20191, KC 20229, KC 20192, KC 20193, KC 20195, KC 20267 and KC 20458 were susceptible. Furthermore, genotypes from Gilan and from West Azarbaijan had the lowest and the highest mean of wi^2, respectively. The distribution of wi^2 values was positively skewed, indicating that smaller values were more abundant than higher values which represents a relative stability in response of genotypes during the evaluation period (Figure 2).

Table 5. Eigen values and vectors of resistance components of local barley accessions in three years of evaluation against barley leaf rust in Gorgan hotspot of the disease.

Resistance components	Principal components		
	First	Second	Third
Disease severity (2015-2016)	0.67	0.64	0.12
Infection type (2015-2016)	0.60	0.71	0.10
Disease severity (2016-2017)	0.76	-0.33	0.48
Infection type (2016-2017)	0.72	-0.43	0.46
Disease severity (2017-2018)	0.81	-0.15	-0.51
Infection type (2017-2018)	0.75	-0.24	-0.56
Eigen values	3.12	1.29	1.04
Variance (%)	51.92	21.49	17.28
Cumulative variance (%)	51.92	73.41	90.69

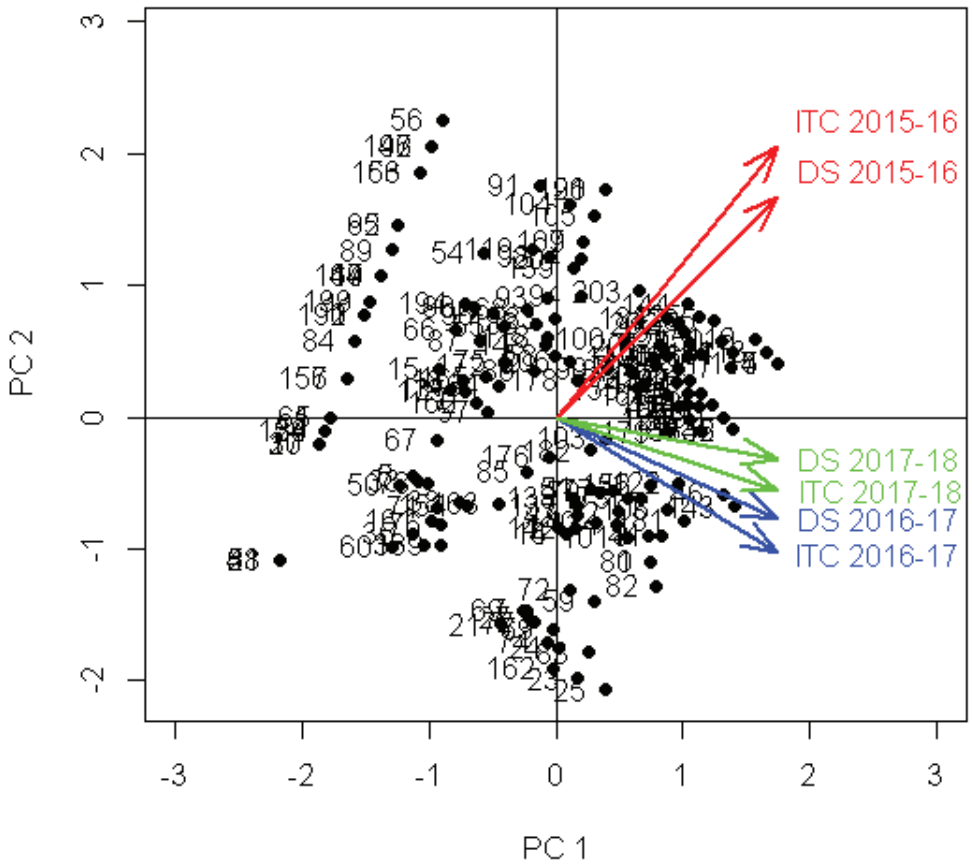

Figure 1. Biplot of the first two principal components based on resistance components of local barley accessions against leaf rust in three years of evaluation in Gorgan hotspot of the disease. *DS and ITC stand for disease severity and infection type, respectively.

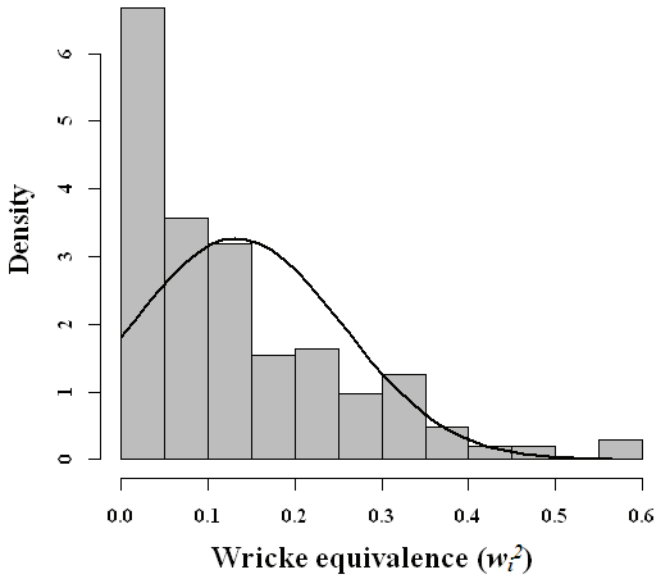

Figure 2. Histogram of Wricke equivalence (w_i^2) calculated based on coefficient of infection in three years of evaluation in local barley accessions against leaf rust in Gorgan hotspot of the disease.

Table 6. Values of Wricke equivalence (w_i^2) calculated based on coefficient of infection in three years of evaluation of local barley accessions against leaf rust in Gorgan hotspot of the disease.

No.	Accession code	w_i^2	No.	Accession code	w_i^2
1	KC 18030	0.22	54	KC 19094	0.10
2	KC 18031	0.30	55	KC 19095	0.05
3	KC 18032	0.30	56	KC 19096	0.42
4	KC 18033	0.25	57	KC 19098	0.14
5	KC 18034	0.00	58	KC 19101	0.09
6	KC 18035	0.00	59	KC 19102	0.25
7	KC 18411	0.19	60	KC 19103	0.06
8	KC 18412	0.13	61	KC 19104	0.04
9	KC 18415	0.32	62	KC 19105	0.12
10	KC 18419	0.09	63	KC 19106	0.11
11	KC 18421	0.17	64	KC 19107	0.09
12	KC 18425	0.06	65	KC 19108	0.04
13	KC 18426	0.00	66	KC 19109	0.04
14	KC 18429	0.04	67	KC 19112	0.00
15	KC 72264	0.01	68	KC 19113	0.11
16	KC 18523	0.25	69	KC 19114	0.04
17	KC 18530	0.03	70	KC 19115	0.02
18	KC 18531	0.01	71	KC 19117	0.05
19	KC 18532	0.09	72	KC 19120	0.08
20	KC 18534	0.03	73	KC 19121	0.19
21	KC 18535	0.03	74	KC 19122	0.12
22	KC 18536	0.07	75	KC 19123	0.13
23	KC 18595	0.32	76	KC 19124	0.10
24	KC 18604	0.45	77	KC 19125	0.08
25	KC 18605	0.33	78	KC 19126	0.02
26	KC 18636	0.33	79	KC 19127	0.11
27	KC 18637	0.04	80	KC 19738	0.23
28	KC 18638	0.03	81	KC 19889	0.23
29	KC 18639	0.04	82	KC 19890	0.29
30	KC 18640	0.03	83	KC 19923	0.28
31	KC 18643	0.03	84	KC 19925	0.04
32	KC 18644	0.43	85	KC 19929	0.04
33	KC 18645	0.07	86	KC 19931	0.32
34	KC 18646	0.04	87	KC 19932	0.02
35	KC 18647	0.10	88	KC 19939	0.05
36	KC 18648	0.14	89	KC 19940	0.13
37	KC 18649	0.22	90	KC 19941	0.05
38	KC 18650	0.14	91	KC 19942	0.23
39	KC 18652	0.06	92	KC 19943	0.33
40	KC 18653	0.05	93	KC 19957	0.39
41	KC 18654	0.17	94	KC 19958	0.55
42	KC 18655	0.08	95	KC 19959	0.12
43	KC 18657	0.05	96	KC 19960	0.19
44	KC 18725	0.03	97	KC 20057	0.33
45	KC 18726	0.04	98	KC 20150	0.14
46	KC 18727	0.16	99	KC 20177	0.12
47	KC 19086	0.11	100	KC 20178	0.08
48	KC 19087	0.03	101	KC 20179	0.22
49	KC 19088	0.09	102	KC 20180	0.07
50	KC 19090	0.00	103	KC 20181	0.09
51	KC 19091	0.03	104	KC 20182	0.23
52	KC 19092	0.16	105	KC 20183	0.34
53	KC 19093	0.03	106	KC 20184	0.20

Table 6 (Continue). Values of Wricke equivalence (w_i^2) calculated based on coefficient of infection in three years of evaluation of local barley accessions against leaf rust in Gorgan hotspot of the disease.

No.	Accession code	w_i^2	No.	Accession code	w_i^2
107	KC 20185	0.02	160	KC 20256	0.16
108	KC 20186	0.12	161	KC 20257	0.05
109	KC 20187	0.31	162	KC 20258	0.14
110	KC 20188	0.01	163	KC 20259	0.46
111	KC 20189	0.03	164	KC 20260	0.12
112	KC 20190	0.01	165	KC 20261	0.18
113	KC 20191	0.00	166	KC 20262	0.24
114	KC 20192	0.01	167	KC 20264	0.31
115	KC 20193	0.01	168	KC 20266	0.11
116	KC 20194	0.01	169	KC 20267	0.01
117	KC 20195	0.01	170	KC 20268	0.16
118	KC 20196	0.40	171	KC 20269	0.16
119	KC 20197	0.10	172	KC 20271	0.22
120	KC 20198	0.39	173	KC 20272	0.19
121	KC 20199	0.39	174	KC 20273	0.02
122	KC 20200	0.14	175	KC 20274	0.12
123	KC 20203	0.07	176	KC 20275	0.07
124	KC 20205	0.06	177	KC 20276	0.07
125	KC 20206	0.14	178	KC 20277	0.11
126	KC 20207	0.07	179	KC 20455	0.10
127	KC 20208	0.12	180	KC 20456	0.03
128	KC 20209	0.04	181	KC 20457	0.14
129	KC 20210	0.12	182	KC 20458	0.01
130	KC 20211	0.07	183	KC 20550	0.29
131	KC 20212	0.02	184	KC 20551	0.25
132	KC 20213	0.04	185	KC 20552	0.11
133	KC 20214	0.06	186	KC 20553	0.03
134	KC 20215	0.23	187	KC 20554	0.05
135	KC 20216	0.05	188	KC 20555	0.34
136	KC 20217	0.13	189	KC 20556	0.20
137	KC 20218	0.02	190	KC 20557	0.05
138	KC 20219	0.03	191	KC 20558	0.05
139	KC 20222	0.30	192	KC 20559	0.05
140	KC 20223	0.11	193	KC 20560	0.06
141	KC 20224	0.07	194	KC 20561	0.07
142	KC 20225	0.04	195	KC 20562	0.02
143	KC 20227	0.22	196	KC 20927	0.39
144	KC 20228	0.21	197	KC 20928	0.07
145	KC 20229	0.00	198	KC 20930	0.03
146	KC 20230	0.33	199	KC 20950	0.02
147	KC 20231	0.09	200	KC 20968	0.02
148	KC 20232	0.30	201	KC 20969	0.20
149	KC 20233	0.03	202	KC 20971	0.19
150	KC 20238	0.05	203	KC 20972	0.28
151	KC 20239	0.19	204	KC 20974	0.56
152	KC 20240	0.13	205	KC 20975	0.19
153	KC 20248	0.24	206	KC 20981	0.55
154	KC 20249	0.21	207	KC 20982	0.07
155	KC 20251	0.02			
156	KC 20252	0.05			
157	KC 20253	0.05			
158	KC 20254	0.04			
159	KC 20255	0.04			

The conformity of matrices of the genetic distances between the studied genotypes calculated form evaluations in each year of experiment was investigated using the Mantel test (Figure 3). Based on these results, all estimated correlation values were significant (despite the small values for most of them). The highest correlation coefficient (r=0.82) was assigned to the comparison of third year assessment with the total evaluations of the three years. The results also showed that the matrices of genetic distances evaluated for the first and third year had the least agreement.

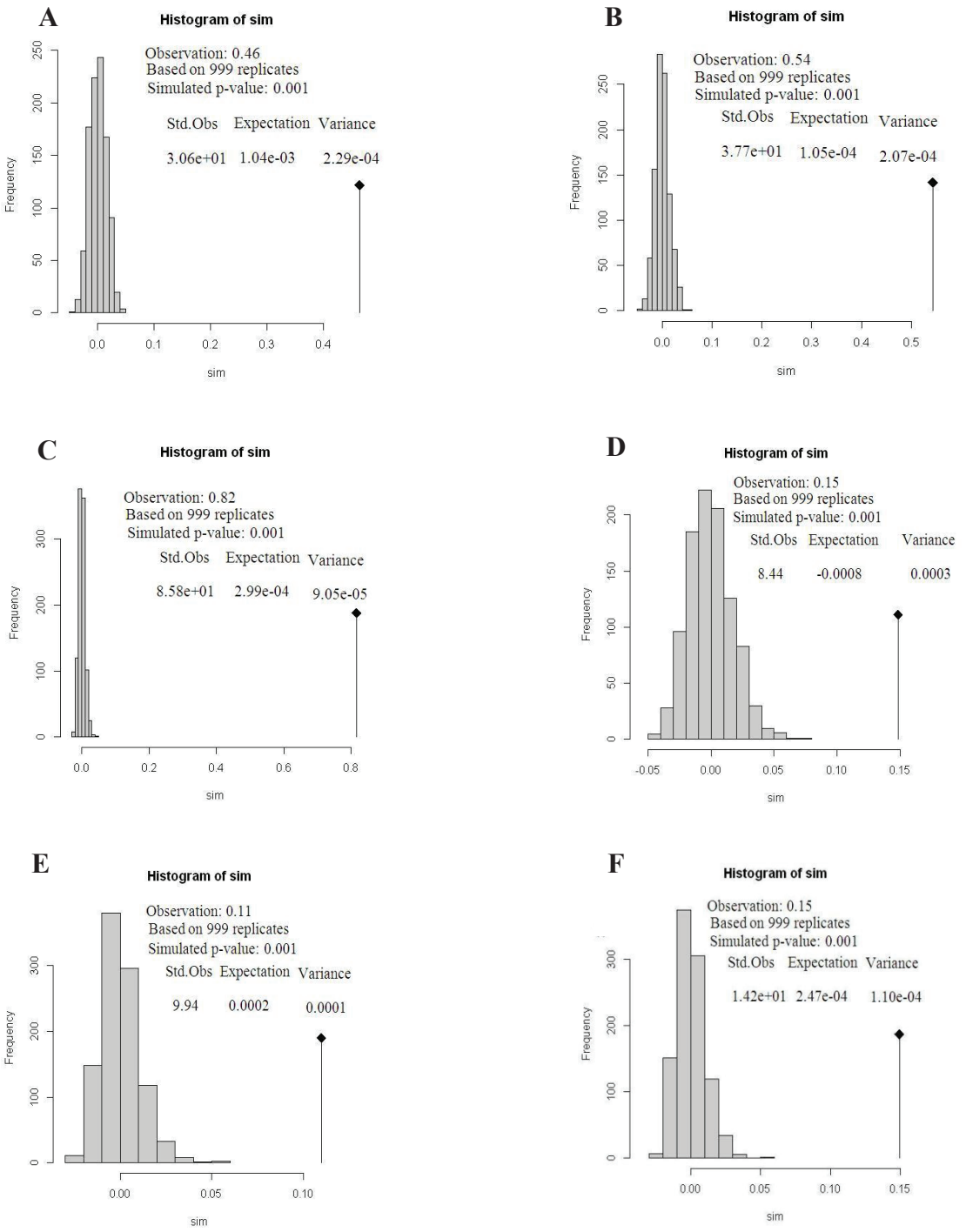

Figure 3. The results of Mantel test for conformity of genetic distances among local barley accessions based on resistance components to leaf rust by using a reference distribution developed through Monte-Carlo approach **A:** years 2015-2016 vs. the whole evaluations, **B:** years 2016-2017 vs. the whole evaluations, **C:** years 2017-2018 vs. the whole evaluations, **D:** years 2015-2016 vs. years 2016-2017, **E:** years 2015-2016 vs. years 2017-2018, **F:** years 2016-2017 vs. years 2017-2018.

The differentiation of the origin of the accessions was investigated using Discriminant Analysis of Principal Components (DAPC) technique. The results showed that the origins of the studied accessions were partially distinguishable based on the first discriminant function along with the horizontal axis (Figure 4). The loading plot of coefficients related to the first discriminant function showed that in general, the disease severity in every three years of the test plays a greater role in this distinction than other components of resistance (Figure 5). Based on the values of posterior probability, the accessions KC 19890, KC 18605, KC 18032, KC 18727 and KC 19889 from West Azarbaijan were assigned to the same origin with a probability higher than 60% (data are not presented). The accessions KC 20188, KC 20190, KC 20194, KC 20191, KC 20975, KC 20192, KC 20193, KC 20195, KC 20206, KC 20228, KC 20185, KC 20212, KC 20198, KC 20199 and KC 20971 from East Azarbaijan were allocated to the same origin with a probability higher than 80%. The accession KC 19124 from Gilan, was assigned to this origin with a probability of 40%. However, the probability of allocationof other accessions originating from Gilan to the same origin was less than 40%. All accessions from Golestan were assigned to this origin with a probability of less than 30%. Also, the allocation probability of the accessions KC 20262, KC 19943, KC 20057, KC 20230, KC 19940, KC 19960, KC 18532, KC 19959, KC 20231, KC 20252, KC 20253, KC 20261, KC 20274, KC 20256, KC 19939, KC 19941 and KC 20271 from Mazandaran to the same origin, was 50% or more.

The studied accessions were also differentiated by cluster analysis using the K method. Ten groups (k=10) were selected based on Bayesian Information Criterion (BIC). The results showed that the germplasm was differently distributed among the groups (Figure 6). Groups 1 and 5 comprised all origins, while other groups only included some of these origins, so that groups 2 and 9 lacked accessions from Gilan and Golestan, group 3 lacked accessions from Gilan, groups 4, 6 and 8 did not contain accessions from Golestan, group 7 did not have accessions from East and West Azarbaijan, and group 10 lacked accessions from East Azarbaijan.

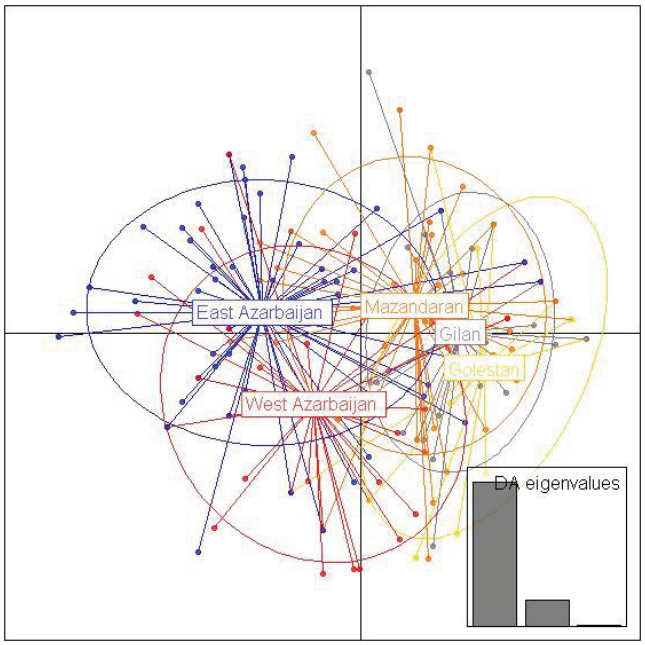

Figure 4. The differentiation of origins of local barley accessions through discriminant analysis of principal components (DAPC) developed by evaluation of resistance components to barley leaf rust in disease hotspot of Gorgan,Iran.

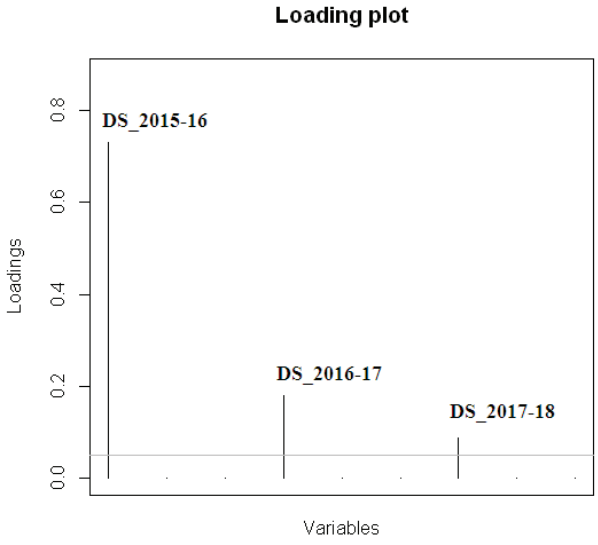

Figure 5. Graphical presentation of the most effective coefficients in discriminant analysis of principal components (DAPC) of origins of local barley accessionsperformed on resistance components to barley leaf rust evaluated within three years in disease hotspot of Gorgan, Iran.
*DS stands for disease severity.

DISCUSSION

Due to the fact that the leaf rust disease has increased in many areas of barley cultivation in the country in recent years and even appeared as an epidemic (Abdolkarim *et al.,* 2011), identification of genetic resources of resistance is very important. In this study, despite the relatively large number of resistant or immune accessions in each year (especially in the second and third year), only four accessions expressed immunity

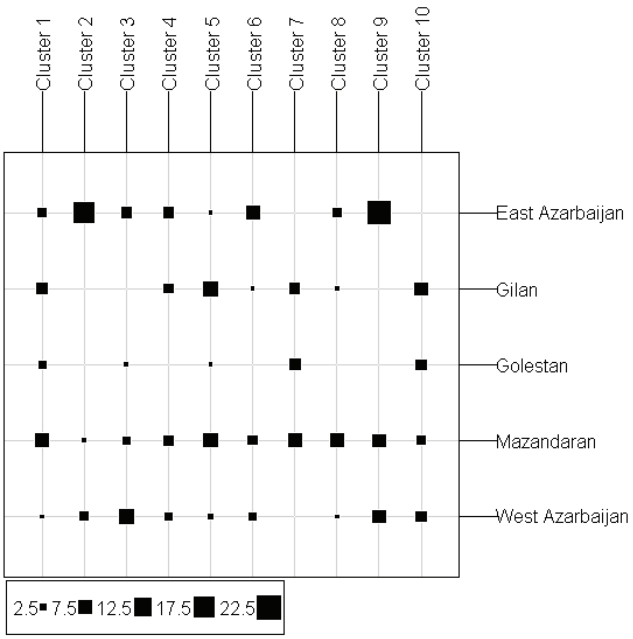

Figure 6. Graphical presentation of the number of members in groups developed by K means clustering method and separated for origin of local barley accessions in evaluation of resistance components to barley leaf rust within three years in disease hotspot of Gorgan, Iran.

response in all three years, and 31 accessions in two years out of three years of experiment. Therefore, the results show that despite the genetic diversity in the studied germplasm, resistance to this disease has been low based on the frequency of effective resistance. The low rates of resistance to barley leaf rust have also been reported by other researchers, however, there are also several reports on high frequencey of resistance to this disease among the germplasm under investigation. In the evaluation of 997 *H. vulgare* accessions by Jin *et al.* (1995), only 58 accessions showed a low infection to the ND89-3 isolate. It has also been observed that among race-specific major resistance genes only *Rh7* showed a complete and effective resistance (Niks *et al.,* 2000), however virulence for this gene has also been reported in Morocco (Parlevliet *et al.,* 1981), the United States (Steffenson *et al.,* 1993) and Israel (Brodney and Rivadeneira, 1996). In the assessment of an international panel of 282 diverse barley entries from 26 countries, depending upon site/season, 2.2-46.1% of the entries appeared very resistant or resistant to leaf rust (Singh *et al.,* 2018). On average, 41% of 213 barley lines from diverse origins carried Adult Plant Resistance to *Puccinia hordei* (APR), of which Chinese germplasm possessed the lowest frequency of APR (Dracatos *et al.,* 2015). One hundred and nine out of 241 Nepalese barley accessions showed promising field resistance to leaf rust (Amgai *et al.,* 2016). Five percent of 820 lines of barley nurseries sourced from the International Centre for Agricultural Research in the Dry Areas (ICARDA) were susceptible to barley leaf rust pathogen at both seedling and adult plant growth stages (Sandhu *et al.,* 2016).

The instability of this kind of resistance has led the plant breeders to seek a more durable type of resistance, such as partial resistance. With this approach, the present study attempted to analyze resistance response of the germplasm based on the components of the resistance in quantitative terms. Correlation analysis showed a close relationship between the two components of resistance including disease severity and infection type. Correlation between the estimates of coefficient of infection in different years was also significant. However, the coefficient of this correlation was not high which could be a sign of racial variation in the area under study.These fingings are contrary to the report of Ziems *et al.* (2017) who observed that the disease response of Australian barley breeding populations in the field was highly correlated across environments and years. These results are also illustrated by other statistical analysis techniques used in this study. For example, in the principal component analysis, the second principal component was able to distinguish the resistance response in 2015-2016 from 2016-2017 and 2017-2018. On the other hand, the third principal component also distinguished resistance response in 2015-2016 and 2016-2017 from resistance response in 2017-2018. This suggests the importance of monitoring the changes in the pathogenicity of the disease in the infected regions. Despite the importance of this issue, there have been few studies in this regard within the country, so that only one study by Abdolkarim *et al.* (2011) has been conducted to identify pathotypes and to investigate virulence on known resistance genes.

CONCLUSION

The results of this research showed that it is possible to identify the genetic resources of leaf rust resistance in Iranian local barley germplasm. Resistant accessions identified in this study can be considered as candidates for use in barley breeding programs. The prerequisite for exploiting these accessions is to examine them more precisely in order to identify the type of resistance in terms of seedling or adult plant and to determine the potential resistance genes that they contain. On the other hand, the results showed that there is a possibility of racial variations in the pathogen population in the infected regions. This indicates the importance of

monitoring these changes and the use of genes with effective resistance in the aforementioned regions.

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