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Identification of sources of resistance to yellow rust in barley landraces grown in the northwest regions of Iran

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ABSTRACT

Barley yellow rust is one of the important barley diseases in the world. However, the importance of this disease has recently increased in Iran. The main aim of the present study was to identify the sources of resistance to this disease in a collection of Iranian barley genotypes provided from the National Plant Gene Bank of Iran (NPGBI). For this purpose, a set of 128 accessions of the north and northwest regions of Iran were assessed in terms of resistance components such as disease severity, infection type and coefficient of infection under natural incidence of the disease in the field of Ardebil Research Station as a hotspot region for barley yellow rust during 2019-2022 cropping seasons. The results indicated a considerable genetic diversity among the tested genetic materials in response to this disease. A moderate correlation was observed between the evaluations of different years. The accessions were separated into five groups using cluster analysis, and the second group with 41 members with the lowest infection coefficient average was identified as the most resistant group. The results of this research showed the high capacity of the collection to identify sources of resistance to barley yellow rust disease. The identified resistant germplasm can be used in breeding programs for resistance to this disease.

Key words: Barley stripe rust, Germplasm, Resistance components, Virulence.

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INTRODUCTION

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop in the world after wheat, rice and maize (Hernandez *et al.,* 2020; Rodríguez-García *et al.,* 2021). The cultivated barley is one of the oldest crops which was domesticated around 10,000 years ago in 'Fertile Crescent' (Middle East) and then moved to the rest of the world (Baik and Ullrich, 2008; Nevo, 2013; Kumar *et al.*, 2020b). Barley is often cultivated in marginal agricultural areas and is considered as an important crop for resource poor farmers in many developing countries (Singh *et al.,* 2019; Singla *et al.,* 2020a; Kanwar *et al.,* 2022b).

Stripe rust of barley caused by *Puccinia striiformis* Westend. f. sp. *hordei* Eriks. & Henn is a fungal foliar disease and occurs in the major barley growing areas (Kumar *et al.,* 2020b). The fungus is considered to be one of the most common pathogens of barley causing yield loss up to 70% (Dubin and Stubbs, 1986; Kumar *et al.,* 2020a; Singla *et al.,* 2020b; Kanwar *et al.,* 2022a). The disease was first reported in Iran in 1947 (Esfandiari, 1947) and is increasing in importance in recent years especially in some parts of the northwest and northeast provinces. The reason for this is attributed to changes in virulence factors in pathogen populations during more recent years (Safavi *et al.,* 2013).

Genetic resistance offers an efficient, inexpensive, sustainable and environmentally-friendly mean of controlling plant diseases including barley yellow rust. (Richardson *et al.,* 2006). Two kinds of stripe rust resistance have been reported in barley: qualitative and quantitative resistance. The qualitative resistance is controlled by single genes and follows gene-for-gene interactions between the host and pathogen and generally observed from the seedling plant stage. This type of resistance is race-specific and confers the plant a complete resistance but has a non-durable nature and could become ineffective in a short period of time due to the selection pressure exerted by extensively used resistant varieties which leads to rapid build up of the disease populations with new virulence to resistance genes (Çelik and Karakaya, 2021; Bai *et al.,* 2022). The quantitative resistance exhibits a polygenic inheritance with continuous variation and is generally observed at the adult plant stage. Quantitative resistance, also termed as partial resistance or slow rusting, is race-nonspecific and delays infection, development and reproduction of the pathogen. Therefore, this type of resistance is incomplete so that host plants are infected but spore production is reduced. Quantitative resistance is highly valued due to higher probability of being stable and durable (Niks and Rubiales 2002; Rothwell *et al.,* 2019).

Plant genetic resources are of great value to improving crops (Hudzenko *et al.,* 2021). The erosion of genetic diversity during domestication and selective breeding has reduced the range of resistance genes and alleles in agricultural cultivars which are normally found in wild crop relatives and landraces. Landraces are characterized by well adaptation to changing climate and or more resistance to abiotic and biotic stresses (Bekele *et al.,* 2019). This study was conducted to identify the resistance sources to barley yellow rust in barley collection of the Iranian National Plant Gene-Bank. This is the first report of the survey in this collection to detect resistance accessions to the disease.

MATERIALS AND METHODS

In this research, 128 accessions provided from the barley collection of the National Plant Gene Bank of Iran were investigated (Table 1). All genetic materials have been originated from the northwest regions of Iran. The investigation was carried out in an augmented experimental design with 8 blocks at the Ardebil Research Station as the hotspot of barley yellow rust disease. For each block, 16 accessions were considered along with Afzal, Bahman, Behrokh and Makoei cultivars as controls (a total of 20 genotypes per block), which were randomly distributed within each block. Each genotype was cultivated in a row with a length of one meter and considering a distance of 30 cm between the rows. Also, for every ten rows of genotypes, one row of Afzal variety (in addition to the controls of the blocks) was cultivated as a spreader of infection. Evaluation of resistance was performed after establishment of the disease and at the time of 100% infection of the flag leaves of Afzal variety (Figure 1). For this purpose, the two components of resistance including infection type and disease severity were recorded. Evaluation of disease severity was based on the Modified Cobb's Scale (Peterson *et al.,* 1948) and evaluation of infection type was performed according to the scale 0 (Immune), R (resistant), MR (moderately-resistant), M (intermediate), MS (moderately susceptible) , MSS (moderately susceptible- susceptible) and S (susceptible) were performed according to Roelfs's method (Roelfs *et al.,* 1992). Then, the coefficient of infection was calculated through the product of disease severity by the transformed values of the infection type, taking into account the values of zero to one (at intervals of 0.2) for each of the infection type classes. The

relationship between assessments in different years was studied by estimating Pearson correlation coefficients. The studied accessions were separated into six groups using K-means cluster analysis. In order to confirm the K-means grouping, the principal component-based discriminant function technique of Jombart *et al.* (2010) was used. Based on the coefficient of infection. Statistical analyzes were performed by SPSS software version 16 as well as coding in R software, version 4.2.1.

Table 1. The barley accessions from National Plant Gene-Bank of Iran used for the study of resistance to yellow rust in Ardebil hotspot during 2019 to 2021.

Accession	Origin	Accession	Origin	Accession	Origin
18030	West Azarbaijan	20197	East Azarbaijan	20562	East Azarbaijan
18032	West Azarbaijan	20198	East Azarbaijan	20927	West Azarbaijan
18033	West Azarbaijan	20199	East Azarbaijan	20928	West Azarbaijan
18034	West Azarbaijan	20200	East Azarbaijan	20950	East Azarbaijan
18411	West Azarbaijan	20203	East Azarbaijan	20968	East Azarbaijan
18412	West Azarbaijan	20204	East Azarbaijan	20975	East Azarbaijan
18429	East Azarbaijan	20205	East Azarbaijan	20981	West Azarbaijan
18595	West Azarbaijan	20206	East Azarbaijan	70102	West Azarbaijan
18603	West Azarbaijan	20207	East Azarbaijan	70103	West Azarbaijan
18604	West Azarbaijan	20208	East Azarbaijan	70104	West Azarbaijan
18605	West Azarbaijan	20209	East Azarbaijan	70105	West Azarbaijan
18652	West Azarbaijan	20210	East Azarbaijan	70106	West Azarbaijan
18653	West Azarbaijan	20211	East Azarbaijan	70107	West Azarbaijan
18654	West Azarbaijan	20212	East Azarbaijan	70108	West Azarbaijan
18655	West Azarbaijan	20213	East Azarbaijan	70109	West Azarbaijan
18656	West Azarbaijan	20214	East Azarbaijan	70110	West Azarbaijan
18657	West Azarbaijan	20215	East Azarbaijan	70111	West Azarbaijan
18725	West Azarbaijan	20216	East Azarbaijan	70113	West Azarbaijan
18726	West Azarbaijan	20217	East Azarbaijan	70114	West Azarbaijan
18727	West Azarbaijan	20218	East Azarbaijan	70115	West Azarbaijan
19889	West Azarbaijan	20219	East Azarbaijan	70116	West Azarbaijan
19890	West Azarbaijan	20222	East Azarbaijan	70117	West Azarbaijan
20150	West Azarbaijan	20223	East Azarbaijan	70118	West Azarbaijan
20177	East Azarbaijan	20224	East Azarbaijan	70119	West Azarbaijan
20178	East Azarbaijan	20225	East Azarbaijan	70120	West Azarbaijan
20179	East Azarbaijan	20227	East Azarbaijan	70121	West Azarbaijan
20180	East Azarbaijan	20228	East Azarbaijan	70122	West Azarbaijan
20181	East Azarbaijan	20238	West Azarbaijan	70123	West Azarbaijan
20182	East Azarbaijan	20239	West Azarbaijan	70124	East Azarbaijan
20183	East Azarbaijan	20240	West Azarbaijan	70125	East Azarbaijan
20184	East Azarbaijan	20455	West Azarbaijan	70126	East Azarbaijan
20185	East Azarbaijan	20456	West Azarbaijan	70134	East Azarbaijan
20186	East Azarbaijan	20457	West Azarbaijan	70135	East Azarbaijan
20187	East Azarbaijan	20458	West Azarbaijan	70138	East Azarbaijan
20188	East Azarbaijan	20551	East Azarbaijan	70139	East Azarbaijan
20189	East Azarbaijan	20553	East Azarbaijan	70140	East Azarbaijan
20190	East Azarbaijan	20554	East Azarbaijan	70141	East Azarbaijan
20191	East Azarbaijan	20555	East Azarbaijan	70155	East Azarbaijan
20192	East Azarbaijan	20556	East Azarbaijan	70485	East Azarbaijan
20193	East Azarbaijan	20558	East Azarbaijan	70487	East Azarbaijan
20194	East Azarbaijan	20559	East Azarbaijan	70488	East Azarbaijan
20195	East Azarbaijan	20560	East Azarbaijan	70491	East Azarbaijan
20196	East Azarbaijan	20561	East Azarbaijan		

Figure 1. Susceptible and resistant accessions of Iranian barley landraces under stripe rust disease incidence in Ardebil hotspot.

RESULTS AND DISCUSSION

The results of ANOVA for the control cultivars (in augmented deign) showed that there was no significant difference between the blocks (results are not presented) and therefore, the recorded values were used for statistical analysis with no correction. Comparison of the frequencies of the infection type in each year showed that among the infection types, MSS had the highest frequency in 2018-2019 and 2020-2021 whereas S was the most frequent in 20192020 (Figure 2). In addition, by comparing three years it was evident that infection types R, MR, M, MS, MSS and S were the most frequent in 2018-2019, 2020-2021, 2019-2020, 20182019, 2020-2021 and 2019-2020, respectively. The average for all resistance components increased from the first year to the second year and decreased from the second year to the third year. In the case of variance, it seems that the infection type, unlike the other two components of resistance, had an almost constant value in different years (Figure 3).

The estimation of the correlation coefficients between the resistance components indicates that in every three years, coefficient of infection had a strong relationship with the other two resistance components, which is reasonable considering that the calculation of coefficient of infection based on infection type and disease severity. However, this relationship was not complete and in different years, changes in the correlation coefficient between coefficient of infection with infection type and disease severity could be observed so that the strongest relationship existed in the second year (Figure 4). The infection type and disease severity also showed a strong relationship so that the correlation coefficient between them varied from 0.78 to 0.90 in different years. It was also apparent that the correlation between coefficient of infection values in the second and third years (0.78) was a little higher than that in the first and second (0.63) and the first and third years (0.64). A similar pattern can be observed for disease severity, but regarding the infection type, the pair-wise correlation coefficients between different years were close to each other.

The studied accessions were separated into five groups using cluster analysis (Table 2). The cluster 2 with 41 members had the lowest average for the coefficient of infection, hence it could be identified as the most resistant group and the cluster 4 with 12 members and the highest average of coefficient of infection, as the most susceptible cluster. The first cluster with 27 members showed higher resistance after the second group and based on the average coefficient of infection, it was placed in the range of moderately-resistant to intermediate class. The fifth cluster showed moderately-resistant reaction on average in the first and third years and a susceptible reaction in the second year, so it was located in the intermediate class. The reaction of the third cluster tended to be susceptible and

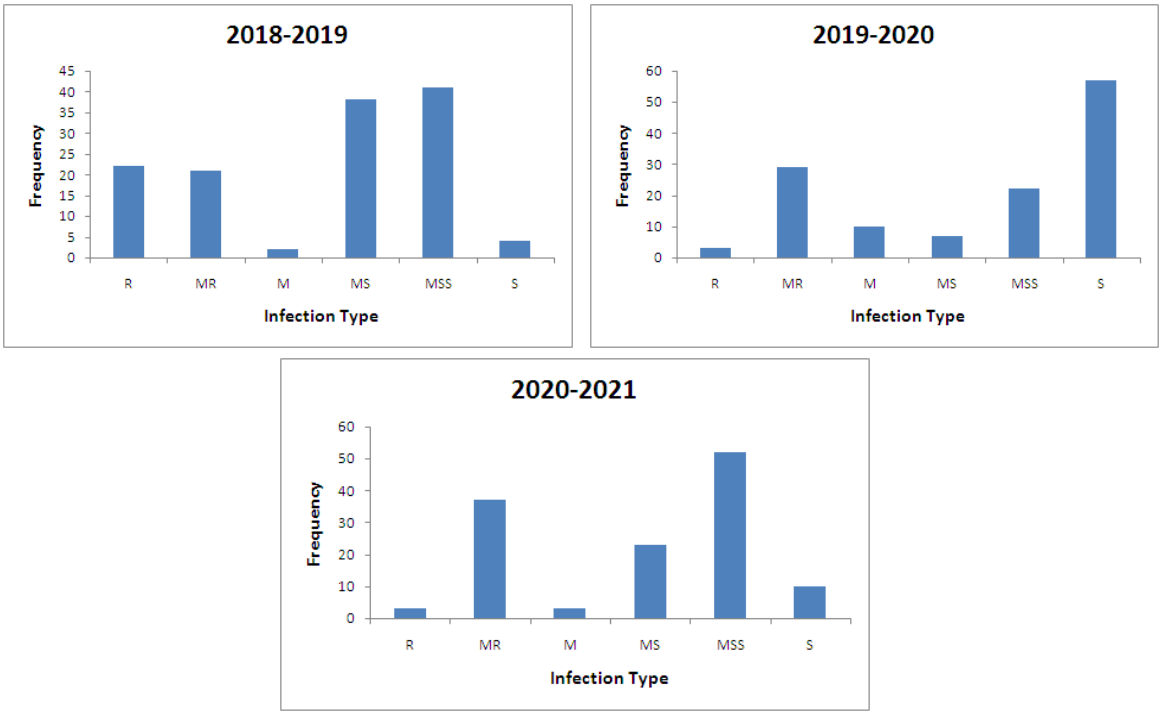

Figure 2. Frequency of infection type of Iranian barley landraces in response to stripe rust evaluated in Ardebil hotspot during 2019 to 2021.
R: Resistant, MR: Moderately resistant, M: Intermediate, MS: Moderately susceptible, MSS: Moderately susceptible-susceptible, S: Susceptible.

could be placed in the moderately-susceptible category.

Table 2. Average coefficient of infection for the groups developed by cluster analysis based on the evaluation of local barley accessions in response to yellow rust in Ardebil hotspot during 2019 to 2021.

Year	Cluster				
	1	2	3	4	5
2018-2019	0.175	0.087	0.332	0.768	0.227
2019-2020	0.441	0.110	0.965	0.992	0.864
2020-2021	0.197	0.109	0.632	0.705	0.328

In order to investigate the resistance reaction of the accessions in more detail, each cluster was further divided to some subgroups. Cluster **1** included five subgroups 1.a, 1.b, 1.c, 1.d and 1.e. Subgroup **1.a** included the accessions that showed R infection type in the first year and other infection types in the second and third years. In this subgroup, the range of disease severity for the MS infection type was from 30 to 50% and for MSS from 40 to 60%. The infection type S also appeared with a disease severity of 60%. KC 18429, KC 18605, KC 20184, KC 20927 and KC 70138 samples were located in this subgroup. Subgroup **1.b** included two accessions, KC 18034 and KC 18654, which showed M infection type in the first and second years and 20MR or 30MS in the third year. The disease severity range for M infection type varied from 30 to 50%. Subgroup **1.c** included KC 18411, KC 18595, KC 70117, and KC 70134 accessions, all of which showed MR infection type (with a range of disease severity from 10 to 40%) in the first and third years and infection type of MSS (with disease severity of 40 or 60 percent) in the second year. Subgroup **1.d** included KC 20180, KC 20216, KC 20223, KC 70107, KC 70108, KC 70115, KC 70126 and KC 70139 accessions, with M infection type (20 or 60% disease severity) or MR (with disease severity 10 or 30%) in one of the years of the experiment and MS infection type (with disease severity from 20 to 60%) or MSS (with disease severity from 40 to 60%) in the other years.

Subgroup **1.e** included KC 18726, KC 18727, KC 20205, KC 20238, KC 20950, KC 20968, KC 20975 and KC 70141 accessions with MS infection type (disease severity from 10 to 40%) and MSS (with disease severity from 30 to 70 percent) appeared in one of the years of the experiment. Two samples KC 20205 and KC 20238 showed MS infection type in all three years.

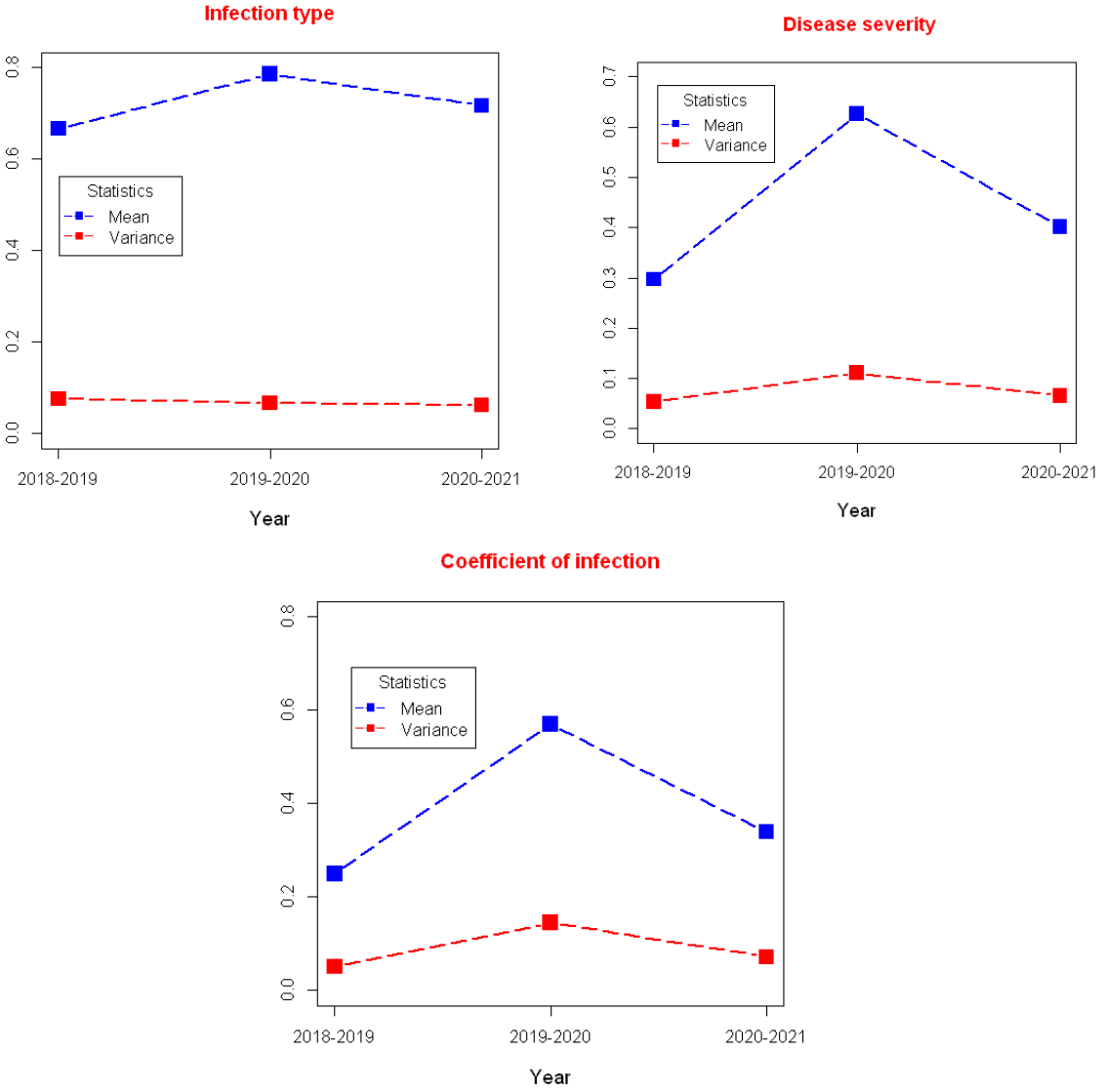

Figure 3. Trend of mean and variance of resistance components of Iranian barley landraces in response to stripe rust evaluated in Ardebil hotspot during 2019 to 2021.

Cluster **2** included three subgroups 2.a, 2.b and 2.c. A total of 32 accessions were placed in subgroup **2.a** showing R, MR or M infection type in two years of the experiment. This subgroup was identified as the most resistant set among all genetic materials studied. The resistance reaction of the members of this group is provided in the Table 3. In this subgroup disease severity range from 10 to 40% for MR infection type, 20 or 30% for MS, 30 or 40% for MSS, and 40% for M. Subgroup **2.b** included KC 18657, KC 20183, KC 20185 and KC 20188, with M infection type (disease severity of 30 or 40%) or MR (with disease severity in the range of 10 to 30%), in two years of the experiment, and MS and MSS infection types (both with disease severity of 30%) in the other year. Subgroup **2.c** included KC 19890, KC 20455, KC 20562, KC 70118 and KC 70491 accessions with 30 M or 20 MR infection type in one year of the experiment and MS infection type (with disease severity of 20 or 30%) or MSS (with disease severity from 30 or 50 percent) in the other years.

Cluster **3** included three subgroups 3.a, 3.b and 3.c. Subgroup **3.a** contained a single member KC 70121 with 20MR-100S-70MSS reaction in the first to third years of the experiment, from left to right. Subgroup **3.b** included ten accessions KC 18604, KC 20182, KC 20190, KC 20191, KC 20198, KC 20206, KC 20207, KC 20209, KC 20210 and KC 20212, all of which appeared MS-S-MSS in the first to third years, respectively from left to right. The severity of the disease for MS infection type ranged from 20 to 40%, for MS infection type, from 60 to 80% for MSS,

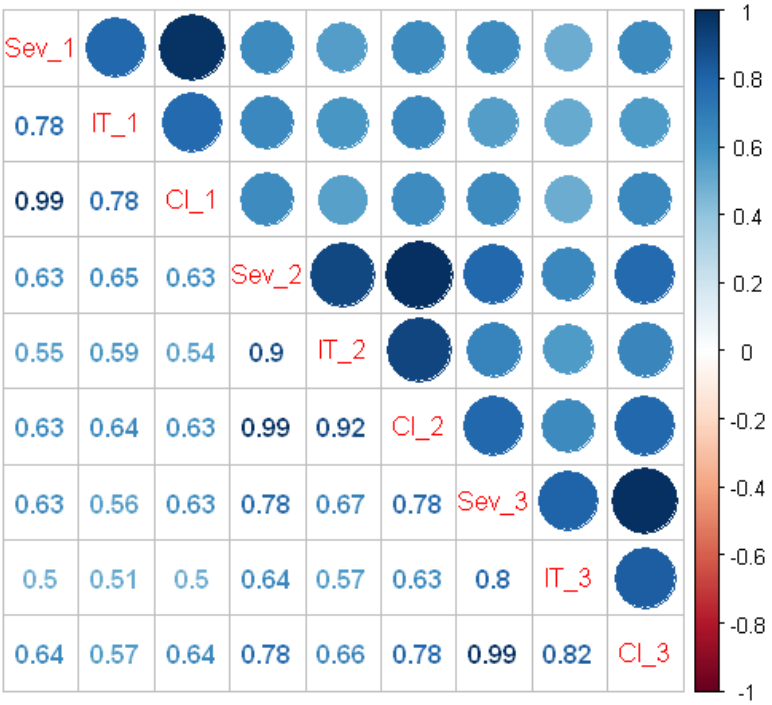

Figure 4. Correlation coefficients between resistance components of Iranian barley landraces in response to stripe rust evaluated in Ardebil hotspot.
IT, Sev and CI correspond to infection type, disease severity and coefficient of infection, respectively.
The year of evaluation is indicated by 1,2 and 3 for 2019, 2020 and 2021, respectively.

Table 3. The resistance reaction of 2.a subgroup members identified as the most resistant set in cluster analysis of barley accessions from National Plant Gene-Bank of Iran in the study of resistance to yellow rust in Ardebil hotspot during 2019 to 2021.

Accession	Reaction	Accession	Reaction	Accession	Reaction
KC 18032	10MR_20MR_40MSS	KC 20215	20MR_20MR_10MR	KC 20558	10MR_R_10MR
KC 19889	20MS_20MR_20MR	KC 20218	R_20MR_10MR	KC 20559	R_R_10MR
KC 20177	R_20MR_20MR	KC 20219	20MS_20MR_R	KC 20560	R_10MR_10MR
KC 20178	R_30MR_20MR	KC 20227	20MR_20MR_20MR	KC 20561	R_20MR_30MS
KC 20179	10MR_30MR_30MR	KC 20239	10MR_20MR_20MS	KC 70120	R_30MR_20MS
KC 20186	R_30M_20MR	KC 20240	20MR_40MR_10MR	KC 70135	R_20MR_20MR
KC 20189	R_20MR_10MR	KC 20456	R_20MR_10MR	KC 70140	20MS_20MR_10MR
KC 20196	10MR_30MSS_10MR	KC 20458	10MR_20MR_20MS	KC 70155	20MR_30MR_20MS
KC 20197	10MR_20MR_R	KC 20554	10MR_20MR_20M	KC 70488	40MSS_20MR_20MR
KC 20200	R_30MS_10MR	KC 20555	R_10MR_10MR	KC 18412	R_40M_20MS
KC 20204	R_20MR_10MR	KC 20556	R_R_10MR		

and 90 or 100% for S. Subgroup **3.c** included twenty accessions which appeared with S infection type (disease severity from 60 to 100%) in one or two years and MSS infection type (with disease severity from 30 to 70%) in other year(s).

Cluster **4** included two subgroups 4.a and 4.b. Subgroup **4.a** included seven accessions that showed S infection type (with disease severity from 80 to 100%) in two or three years and MSS infection type (with disease severity from 60 to 80%) in the year(s). Subgroup **4.b** included five accessions, all of which showed the MSS-S-MSS infection type, from left to right, corresponding to the first to third years of the experiment, showing the disease severity of 60 to 80% for MSS, and 100% for S infection type.

Cluster **5** was also divided into two subgroups 5.a and 5.b. Subgroup **5.a** had four accessions showing infection type R or 20 MR in one or two years of the experiment and infection types 30 MS, MSS (with disease severity from 40 to 80%) or S (with disease

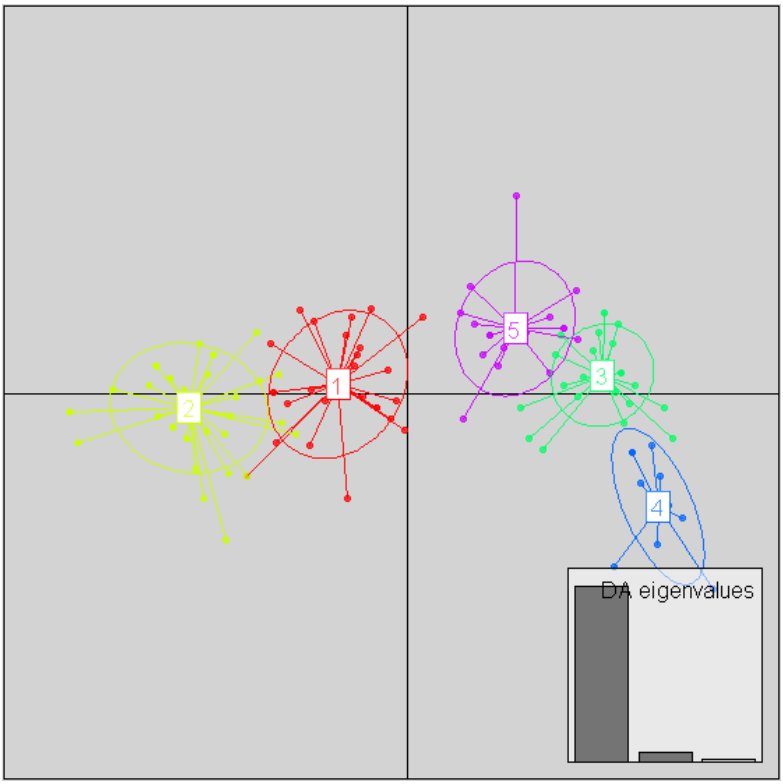

Figure 5. Distinction of the clusters of Iranian barley landraces by discriminant functions based on coefficient of infection in response to stripe rust evaluated in Ardebil hotspot during 2019 to 2021.

severity of 90 or 100%) in the other year(s). Subgroup **5.b** containing 13 accessions exhibited MS infection type (with disease severity of 20-40%) or MSS (with disease severity of 20-70%) in two years of the experiment and S infection type (with disease severity of 80-100%) in some years.

The clusters were well separated in the biplot of the discriminant functions showing the least interference between the groups (Figure 5). The second group (as the most resistant group) was located on the left side of the diagram and the fourth group (as the most susceptible group), on the right side of the diagram, completely distinct from other groups. The first cluster was also located close to it and the fifth group (the intermediate class) in the middle of biplot among other groups. Therefore, the range of resistance to susceptibility could be well identified in the horizontal axis of the discriminant function, and these observations validate the results of cluster analysis and the developed groups, thereof.

The results of this research showed the appropriate capacity of the barley collection of the National Plant Gene Bank of Iran as sources of resistance to barley yellow rust, so that among the evaluated accessions, 33.6%, 25% and 31.3% appeared as resistant or moderately-resistant in three years of the experiment, respectively. These ratios are comparable with other studies, for example in the research carried out by Visioni *et al.* (2018), 16.5% to 62% (depending on the race used in the seedling stage) of the studied barley genotypes, including released cultivars, advanced breeding lines and landraces, showed resistance. In the research performed by Verma *et al.* (2018), only six genotypes out of the 336 studied barley genotypes were resistant to all five races used in the seedling and adult plant stages. Also, according to Gyawali *et al.* (2021), less than six percent of 336 barley genotypes showed immunity in the adult plant stage, and about 25 percent were resistance. Thus, the present results indicate the suitability of local barley populations as sources of resistance to barley yellow rust disease. A similar research also shows the existence of sources of resistance to this disease in indigenous genetic resources (such as Karkee *et al.,* 2022)

Observing a different proportion of resistance during different years (or in different disease hotspots) is common in the evaluations of genotype reactions to the disease and can indicate a change in the dominant race of the disease in the region, for example in the research conducted by Visioni *et al.* (2018), 46.7% and

27.9% of genotypes showed resistance in 2013 and 2014, respectively in Durgapura district and 77% in Karnal district in 2014 at the adult plant stage. Hence, the differentiation of the accessions in the experimental years and the observed changes in their response to the disease in the present study can indicate racial changes in the pathogenic populations in the tested area. The existence of race for *Puccinia striiformis* was first reported by Allison and Isenbeck (1936) based on specificity on wheat cultivars. Extensive studies on pathogenicity specificity were conducted in Europe between 1930 and 1960, mainly on wheat (Zadoks, 1961; 1965). Chen and Penman (2005) identified 74 races of *Puccinia striiformis* f. sp. *hordei*. The highest number of new races was identified in 1995 and 1996 (Chen and Line, 2001), which could be responsible for the severe damage to barley production in 1997 and 1998 in the United States of America. Seven geographic regions in the United States have been defined based on the occurrence and virulence of *Puccinia striiformis* and other factors (Line and Qayoum, 1992). The existence of different pathotypes of *Puccinia striiformis* f. sp. *hordei* has also been reported in Iran. Safavi *et al.* (2013) identified a total of ten pathotypes of barley yellow rust, of which seven pathotypes were reported for the first time in the world. The isolates investigated in this research were collected from the provinces of Ardabil, Khorasan, Fars, West Azerbaijan, Kurdistan, Golestan and Khuzestan. Therefore, considering the genetic diversity of this fungus in different regions, it is suggested that in future studies, the germplasms are evaluated in several regions where this disease is more spread and prevalent.

The results of present study also revealed the diversity of resistance to barley yellow rust in the studied germplasm. The accessions with different infection types and various levels of disease severity were separated into different clusters which could be selected for building diverse genetic population of the resistance sources to the disease. Two accessions, KC 20556 and KC 20559, which showed R infection type at least in two years, can be investigated to identify the major R genes. Accessions KC 20179, KC 20215 and KC 20227 showed MR infection type in all three years with disease severity in the range of 10 to 40%, which can be investigated to identify the adult plant resistant (APR) type. Also, KC 70118 (with 20MS-20MR-20MS reaction) showed a stable level of disease severity over three years, which could be considered for studies related to APR. Considering the complementary reaction of two accessions KC 20220 (R-30MS-10MR) and KC 20561 (R-20MR-30MS) in the second and third years, the presence of different resistance genes in these accessions can be investigated for pyramiding objective.

CONCLUSION

Overall, the results of this research led to the identification of barley germplasm with effective resistance to yellow rust disease, which can be used as genetic sources of resistance in barley breeding programs. Also, considering the dynamic nature of pathogenicity in the pathogen population, it is recommended to continue the search for new sources of resistance in the barley collection of the National Plant Gene Bank of Iran. The evaluated resistance components showed a strong relationship, but their correlation was not complete, which indicates that each one of them can represent a different aspect of resistance, so it is recommended to conduct future research based on the evaluation of different types of resistance components. Racial changes in the pathogenic population were evident based on the change in the reaction of the studied accessions, so it is better to select for effective resistance sources by considering this issue and conducting experiments in different disease hotspots and during different years. It is also recommended to monitor racial changes of the pathogen using differential varieties carrying different R genes and to conduct such studies together with germplasm screening research. Also, it will be useful to carry out greenhouse research to differentiate types of resistance and to identify the genes responsible for resistance in the evaluated genetic materials.

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