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Research Article

Screening of dryland bread wheat genotypes against yellow rust through greenhouse and multi-environmental trials

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Abstract: Yellow rust of wheat caused by *Puccinia striiformis f.* sp. *tritici* is one of the most important wheat diseases that threaten its production. Host resistance is the most economical and healthiest method of yellow rust management. In this study, slow rusting resistance parameters including infection coefficient, disease severity as well as reaction type were recorded for 48 rain-fed wheat genotypes along with sensitive control in 6 rain-fed environments. The field experiment was done for two consecutive years in three distinct geographical regions including Ardebil, Zarghan, and Mashhad. The additional screening test was established in greenhouse conditions. Results showed genetic variability among studied germplasms in resistance against yellow rust. In this research, G1, G04, G05, G06, G20, G21, G32, G33, G39, and G45 out of the studied genotypes had resistant and stable reactions across years and locations. Ward clustering algorithm produced three heterotic groups which can be utilized in yellow rust breeding programs through parental selection for the construction of a yellow rust mapping population. Differential genotypes testing resulted in "6E142A +, Yr27", "38E158A +, YR27" and "134E150A +, YR27" isolates which belonged to Ardebil, Mashhad as well as Zarghan regions respectively. The resistance reaction in the seedling stage varied from that found in the field state which indicated the existence of adult plant resistance genes in their genome.

Keywords: wheat, *Puccinia striiformis*, Seedling resistance, rainfed environments, Adult plant resistance

Introduction

Yellow (stripe) rust is one of the most devastating wheat diseases worldwide caused by the biotrophic basidiomycetous fungus *Puccinia striiformis* Westend. f. sp. *tritici* Eriks. (PST) (Chen 2005; Hovmøller *et al*. 2010; Prins *et al*. 2016; Lei *et al*. 2017). Wheat plants can be infected at any time

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from one-leaf stage seedlings to mature-stage green plants (Chen, 2005). The disease is traditionally managed via the cultivation of wheat varieties that harbor effective major race-specific resistance (R) genes. Although large-scale breeding efforts to improve disease resistance have controlled wheat diseases (Singh *et al.*, 2016), such a strategy is however, seriously challenged by the evolution of new resistance-breaking races of PST (Cantu *et al.*, 2013). For instance, the appearance of PST races that overcome widely deployed R genes (such as *Yr2*, *Yr9*, *Yr17*, and *Yr27*) have resulted in devastating pandemics (Welling, 2011). Varieties carrying single resistance genes often become susceptible in a relatively short period (5–8 years) following the appearance of new races of the pathogen (Tomar *et al.*, 2014). In recent years, the emergence of new more aggressive races of PST has led to increased concerns over the disease. Although the disease occurs mainly in cool conditions (Roelfs *et al.*, 1992), these races are of more virulence genes and able to adapt to warmer temperatures compared to most former races (Hovmøller *et al.*, 2010). Because of the wind-borne nature of PST ant the spread its spores over very long distances (Brown and Hovmøller, 2002), these new races are potential threats to global wheat production and food security (Milus *et al.*, 2009). The cultivation of wheat cultivars resistant to yellow rust is the most efficient, economical, and eco-friendly approach to controlling this rust (Chen, 2013). More than 80 genes (*Yr* genes) conferring resistance to yellow rust have been reported in wheat and most of them have been applied in breeding programs aimed at yellow rust resistance development in wheat (Zeng *et al.*, 2014; Yuan *et al.*, 2018; Tahir *et al.*, 2020). The effectiveness and durability of stripe rust resistance can be achieved through the use of proper genes and via their pyramiding in a cultivar (Simons *et al.*, 2011; Pouralibaba *et al.*, 2021). Furthermore, the ever-challenging evolution of new races armed with novel virulence genes against the introduced resistance genes (Afzal *et al.*, 2010; Hubbard *et al.*, 2015) has led to the increased emphasis on the application of the cultivars that harbor a set of genes conferring race-specific resistance and non-race-specific resistance and are expected to represent a long-term strategy for yellow rust management (Singh *et al.*, 2011; Chen 2013; Chen *et al.*, 2013; Hulbert and Pumphrey, 2014). The slow-rusting or high-temperature adult (HTAP) resistance genes (such as *Yr18/Lr34*, *Yr29/Lr46*, and *Yr46*) are examples of non-race-specific resistance genes that have provided durable resistance for over 50 years (Chen *et al.*, 2013; Singh *et al.*, 2014). The lines with HTAP or slow-rusting genes are susceptible at the seedling stage when the temperature is low, however, these lines get resistant at the adult plant stage when it gets warm (Tahir *et al.*, 2020). Seedling resistance is expressed throughout plant life and is believed to be the phenotype of race-specific resistance genes and is subject to breakdown. Adult plant resistance genes are ineffective at the seedling stage, but expressed at the adult plant stage are effective in a race-non-specific manner and thus are usually found to provide durable resistance to stripe rust (Brar *et al.*, 2017). Most of these cultivars are characterized by their ability to retard and slow down the onset and development of disease during an epidemic in the field. Consequently, these cultivars can reduce and restrict the rate of disease progression and minimize the amount of the disease in the infected tissues, irrespective of their type of reaction to yellow rust infection. This type of resistance has been earlier defined by Parlevliet (1976), as a partial resistance and/ or an adult plant resistance (APR), which is assumed to be more stable and more durable, compared to other forms of genetic resistance (Boulot, 2007). Such resistance was also identified as a polygenic, non-race-specific (general) resistance (Boulot and Gad-Alla, 2007). Thus, it has a permanent effect against a wide range of pathogen races, hence it has been little or not affected by a sudden race changing or evolving in rust pathogen populations (Miedaner and Korzun, 2012). It presumably, lasts longer and remains effective over a wide range of environmental conditions for many years. Therefore, it is regarded as more durable than other types of resistance (Broers and Parlevliet, 1989; Boulot, 2007). Considering the necessity of a continuous search for cultivars resistant to yellow rust, this research was performed with some wheat cultivars seeded in the fall.

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Materials and Methods

Plant material

A suite of 48 bread wheat genotypes along with one susceptible genotype as control (Table 1) were inspected in both field and greenhouse conditions.

Preparation of pathogen inoculum and greenhouse experiment

For the mass production of rust inoculum, the seed of the susceptible cultivar of bread wheat, Bolani, were grown in 6 cm plastic pots. Ten-day-old seedlings with the first leaf fully emerged were wetted using distilled water amended with 1 drop of tween20 oil per liter (Knott, 1988). The seedlings were immediately inoculated using a uredospore sus pension made with industrial oil Saltrol 170 with the final intensity of 0.1 g uredospore's ml^{-1}. The seedlings were misted again, covered with transparent plastic pockets, and incubated at 10 °C in the dark for 48h. Then the seedlings were transferred into greenhouses (18 °C, 16:8 h L: D photoperiod, 10–15 lux. The collection of uredospore was begun on the 15th day after inoculation, repeated in intervals of 3 days, and terminated when the seedlings were dead. The collected spores were stored in a freezer at-17 °C.

Ten seeds of each wheat line/cultivar were grown in 6 cm plastic pots and grown in a greenhouse at 18°C. After 10 days, the seedlings were inoculated with the spores of the existing rust races (Table 2) using a small hand sprayer at the growth stage of the emergence of the first seedling leaf. The inoculated seedlings were incubated under standard conditions (dark and 10 °C, RH %100) for penetration and development of *P. striiformis* f. sp. *tritici*. After 17 days the reaction of the seedlings to yellow rust disease was recorded according to a 0–4 scale (McIntosh *et al.*, 1995). Based on this scale, the cultivars of the reaction type 0–2 are considered as resistant and 3–4 reaction type cultivars are known as susceptible (McIntosh *et al.*, 1995).

Multi-environmental field trials

Field experiments were carried out in three distinct regions including Ardebil, Zarghan, and Mashhad for two consecutive years. Seven-gram seed of each wheat line/variety was grown in two 1m long lines on the top of the row. For pathogen establishment and distribution a highly susceptible cultivar Bolani was planted around the nursery. The inoculation of the wheat plants was started just after tillering to flag leaf stage using a mixture of a regional isolate of yellow rust uredospores and talc powder (1: 3) in a backpack atomizer sprayer and repeated 3 times. The recording of disease progression was carried out three times after the appearance of disease symptoms on flag leaf in 5-7 day intervals and the percentage of infected leaf area (ILA, 0-100%) was recorded following modified Cubb's method (Peterson *et al*., 1948). Moreover, the plant reaction to yellow rust infection was typified following the methodology applied by Roelfs *et al.* (1992) where five types of plant reactions have been identified and symbolized as resistant (R), moderately resistant (MR), moderate (M), moderately susceptible (MS), and susceptible (S). The coefficient of infection was calculated by using Pathan and Park, 2006 method.

Results

Combined analysis of slow rusting attributes

As shown in Table 2, adult plant of 49 bread wheat varieties has different reactions against *P. striiformis* isolates across studied rainfed regions. Disease severity (DS), reaction type (RT), and coefficient of infection (CI) were addressed for each genotype (Table 2). In the Zarghan region, G01 and G05 had resistant-type reactions across two consecutive years and their disease severity varied from 20 to 40. Also, G04, G32, G33, and G39 out of the studied genotypes possessed R reaction in the year 2017-18 along with MR reaction in the year 2018-19. In the Mashhad region, there was no R-type genotype in any of the years and some genotypes had MR reactions across the years. For instance, G01, G04, G05, and G20 had MR reactions across two years. In this research, like the Mashhad region, there were not any R genotypes in the Ardebil region throughout the studied years. While G01, G05, G20, G29, G32, G33, G38, and G39 had MR reactions in both years in the Ardebil region.

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Table 1 Pedigree of rainfed wheat genotypes used in this study and reactions to strip rust races at the seedling stage.

NO	Origin	Pedigree	Seedlings infection type against races		
			38E158A +, YR27	134E150A +, YR27	6E142A +, Yr27
1	Iran	ALTAY//JUN/BOMB IRW 05-06-36-OMAR-OMAR_0MAR	0;	0	0;
2	Iran	ALTAY//JUN/BOMB IRW 05-06-36-OMAR-OMAR_0MAR	3	3	3
3	Iran	PAVON (dwarf)/KAUZ (tall) IRW 05-06-84-OMAR-OMAR_0MAR	3	;2	3
4	Iran	CROC-1/AE.SQUARROSA (205)// KAUZ/3/SASIA/4/CHEN/ AEGILOPS SQUARROSA (TAUS)//BCN /3/VEE#7/… IRW 05-06-221-OMAR-OMAR_0MAR	2 + C	3	2 + C
5	Iran	ZARGANA-3//JUN/BOMB IRW 05-06-333-OMAR-OMAR_0MAR	3	3	3
6	Iran	SOROCA//SAULESKU #44/TR810200 IRW 05-06-171-OMAR-OMAR_0MAR	3	3	3
7	Iran	SOROCA//SAULESKU #44/TR810200 IRW 05-06-171-OMAR-OMAR_0MAR	3	3	3
8	Iran	SERI 82/SHUHA'S'//GRU90-204782/3/SARDARI/KAU"s"/NAO IRW 05-06-210-OMAR-OMAR_0MAR	3	3	3
9	Iran	ALTAY/GAHAR IRW 05-06-41-OMAR-OMAR_0MAR	3	3	3
10	Iran	NGDA146/4/YMH/TOB//MCD/3/LIRA/5/F130L1.12/ 6/PELSART /3/DONG87//TJB368.251/BUC/4/RSK/NAC IRW 05-06-138-OMAR-OMAR_0MAR	3	3	3
11	Iran	NGDA146/4/YMH/TOB//MCD/3/LIRA/5/F130L1.12/ 6/PELSART /3/DONG87//TJB368.251/BUC/4/RSK/NAC IRW 05-06-138-OMAR-OMAR_0MAR	3	3	3
12	Iran	P8-8/LLFN/3/BEZ/NAD//KZM/4/BB//CC/CNO*2/3/TOB156/BB/5/ PF8215/6/F134.71/NAC/7/SARDARI-HR86 IRW 05-06-355-OMAR-OMAR_0MAR	3	3	3
13	Iran	GAHAR/3/SKAUZ/PASTOR//PASTOR*2/OPATA IRW 05-06-145-OMAR-OMAR_0MAR	3	3	3
14	Iran	GAHAR/3/SKAUZ/PASTOR//PASTOR*2/OPATA IRW 05-06-145-OMAR-OMAR_0MAR	3	3	3
15	Iran	KOHDASHT//37032 TURKEY/DARI-8 IRW 05-06-149-OMAR-OMAR_0MAR	3	3	3
16	Iran	BEZ/ALTAY IRW 05-06-14-OMAR-OMAR_0MAR	3	3	3
17	Iran	BEZ/ALTAY IRW 05-06-14-OMAR-OMAR_0MAR	3	3	3
18	Iran	Azar-2/Ardabil 82 - 33 IRBW07-23-54-20 IRBW07-23-54-20-0SAR-0SAR_0MAR	3	3	3
19	Iran	Sardari/Ardabil 82 - 33 IRBW07-23-54-36 IRBW07-23-54-36-0SAR-0SAR_0MAR	3	3	3
20	TCI	"ATTILLA//VORONA/TR810200	2 + C	;2	3
21	TCI	MV14-2000//SHARK/F4105W2.1	0;	;2	3
22	Iran	RAN/NE701136//CI13449/CTK/3/CUPE/4/SXL/VEE/5/1D13.1/MLT//TRK13/3/PKL70/ LIRA/6/5299 IRW08-540-0Mar-0Mar	3	3	3
23	Iran	37025 Turkey/Sabalan//AKSEL IRW08-291-0Mar-0Mar	3	0	2C
24	Iran	RAN/NE701136//CI13449/CTK/3/CUPE/4/SXL/VEE/5/1D13.1/MLT//TRK13/3/PKL70/ LIRA/6/5299 IRW08-540-0Mar-0Mar	3	0;	2C
25	Iran	Altay/3/PTZ NISKA/UT1556-170//UNKNOWNT IRW08-076-0Mar-0Mar	2CN	0;1	2C
26	Iran	Sabalan//Fenkang/Sefid IRW08-102-0Mar-0Mar	3	;1C	3
27	Iran	Fgs/KATIA1 IRW08-222-0Mar-0Mar	3	3	2C
28	Iran	Fgs/Azar-2 IRW08-220-0Mar-0Mar	3	;2	3
29	Iran	VORONA/HD24-12//GUN/3/Tam 200/Kauz IRW08-598-0Mar-0Mar	0	0;	3
30	Iran	Dari-4/5/Cbc//No/Inia/3/Lfd/4/…./6/ERYT5678-F134.71/NAC//ZOMBOR IRW08-643-0Mar-0Mar	3	0	3
31	Iran	Fenkang/Sefid/6/RAN/NE701136//CI13449/CTK/3/CUPE/4/SXL/VEE/5/1D13.1/MLT// TRK13/3/PKL70/LIRA IRW08-250-0Mar-0Mar	2CN	0	3
32	Iran	Fenkang/Sefid/6/RAN/NE701136//CI13449/CTK/3/CUPE/4/SXL/VEE/5/1D13.1/MLT// TRK13/3/PKL70/LIRA IRW08-250-0Mar-0Mar	3	3	2CN
33	Iran	WESTON/VEE/6/RAN/NE701136//CI13449/CTK/3/CUPE/4/SXL/VEE/5/1D13.1/MLT// TRK13/3/PKL70/LIRA IRW08-323-0Mar-0Mar	3	0	3
34	Iran	ICAMOR-TA04-68//SHARK/F4105W2.1 IRW08-353-0Mar-0Mar	3	0	3
35	Iran	RAN/NE701136//CI13449/CTK/3/CUPE/4/SXL/VEE/5/1D13.1/MLT//TRK13/3/PKL70/ LIRA/6/Bayrak tar IRW08-538-0Mar-0Mar	3	0;	3
36	Iran	PAVON DWARF/Azar-2 IRW08-151-0Mar-0Mar	3	0;	3
37	Iran	Sardari/Azar-2 IRW08-133-0Mar-0Mar	3	0	3
38	Iran	100 ZHONG 257//CNO79/PRL/3/OK82282//BOW/NKTT/6/RAN/ NE701136//CI13449/CTK/3/CUPE/4/SXL/VEE/5/1D13.1/MLT//TRK13/3/PKL70/LIRA IRW08-335-0Mar-0Mar	0;C	0	2C
39	Iran	Trakia//Maga"s"74/Mon"s"/3/Shahi/4/91-142 a 61/3/F35.70/MO73//1D13.1/MLT IRW08-232-0Mar-0Mar	3	0	01CN
40	Iran	Bayrak tar/4/DONSKAYA POLUKARLIKOVAYA/OLVIA /3/2*AGRI/BJY//VEE IRW08-126-0Mar-0Mar	3	0;	3
41	Iran	G.B	3	0;	3
42	Iran	G.B	3	0	3
43	Iran	Sardari	3	2 + C	3
44	Iran	Azar 2	3	3	3
45	Iran	Tak-ab	2C	0;	2C
46	Iran	Baran	3	3	3
47	Iran	Hashtrood	3	3	3
48	Iran	Sadra	3	3	3
49	Bolani	(Susceptible Check)	3	3	3

[A] Infection types were determined according to a 0 to 4 scale (McIntosh *et al*. 1995) and ';', 'c' and 'n' indicate a fleck reaction, chlorosis and necrosis, respectively. Plus or minus signs signify larger or smaller pustule variations within an accepted infection type class.

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Table 2 Field-based assessment of slow rusting resistance parameters to yellow rust during 2017-19.

NO	2017-18						2018-19						
	Zarghan		Mashhad		Ardebil		Zarghan		Mashhad		Ardebil		
	DS & RT	CI	APR	CI	DS & RT	CI	DS & RT	CI	DS & RT	CI	DS & RT	CI	ACI
1	40R	8	10MR	4	20MR	8	70R	14	60M	36	30MR	9	13
2	60M	36	50M	30	90MSS	81	80S	80	100S	100	80MSS	72	67
3	50M	30	40M	24	80MSS	72	80MSS	72	60MS	54	70MSS	63	53
4	40R	8	10MR	4	50MS	45	30MR	12	40MR	16	40MR	16	17
5	20R	4	20MR	8	30MR	12	40R	8	30MR	12	30MR	12	9
6	40MR	16	30MR	12	30M	18	40R	8	50MS	45	40M	24	21
7	70MS	63	70S	70	80MSS	72	80MSS	72	100S	100	80MSS	72	75
8	70MS	63	50MS	45	80MSS	72	80MSS	72	100S	100	70MSS	63	69
9	70MS	63	60MS	54	80MSS	72	80MSS	72	100S	100	70MSS	63	71
10	90MS	81	100S	100	100S	100	80MSS	72	100S	100	60MSS	54	85
11	80M	50	90S	90	100S	100	80MSS	72	100S	100	60MSS	54	78
12	60M	36	50MS	45	80MSS	72	70MSS	63	70MS	63	50MS	45	54
13	50MR	20	70MS	63	80MSS	72	70MSS	63	90S	90	70MSS	63	62
14	50MR	20	60MS	54	80MSS	72	80S	80	100S	100	60MSS	54	63
15	60MR	24	50MS	45	70MSS	63	80MSS	72	100S	100	70MSS	63	61
16	70MR	28	60MS	54	90MSS	81	100S	100	100S	100	70MSS	63	71
17	90MSS	81	90S	90	100S	100	90S	90	100S	100	100S	100	94
18	60M	36	60MS	54	90MSS	81	80MSS	72	90S	90	80MSS	72	68
19	60MS	54	50MS	45	90MSS	81	80MSS	72	100S	100	80MSS	72	71
20	5MR	2	20MR	8	10MR	4	10MR	4	10MR	4	10MR	4	4
21	60MR	24	30MR	6	20MR	8	60M	36	0	0	10MR	4	13
22	50M	30	70MS	63	70MSS	63	80S	80	100S	100	70MSS	63	67
23	60MS	54	50M	30	70MSS	63	80MSS	72	100S	100	70MSS	63	64
24	60MS	54	50MS	45	80MSS	72	100S	100	100S	100	50MSS	45	69
25	40MR	16	20M	12	60MS	54	60MS	54	70MS	63	50MSS	45	40
26	60MS	54	50MS	45	60M	36	80S	80	70MS	63	70MSS	63	57
27	50MS	45	70S	70	50M	30	70S	70	90MS	81	80MSS	72	61
28	70MS	63	30M	18	70MSS	63	80S	80	90S	90	50M	30	57
29	50MR	20	50MS	45	40MR	12	60M	36	80MS	72	40MR	16	34
30	70MS	63	80S	80	70MSS	63	70MSS	63	90S	90	50MS	45	67
31	60MS	54	70MS	63	90MSS	81	70MSS	63	80S	80	60MSS	54	66
32	20R	4	20M	12	40MR	12	30MR	12	40M	24	40MR	16	13
33	20R	4	30M	18	40MR	12	40MR	16	50MS	45	30MR	12	18
34	60MS	54	80MS	72	90MSS	81	80MSS	72	100S	100	50MS	45	71
35	70M	42	60MS	54	90MSS	81	70MSS	63	90S	90	60MS	54	64
36	70MS	63	70MS	63	100S	100	80S	80	100S	100	70MS	63	78
37	80MS	72	90S	90	100S	100	100S	100	100S	100	100S	100	94
38	70MS	63	30M	18	40MR	16	100S	100	40MR	16	30MR	12	38
39	20R	4	-	-	20MR	8	30MR	12	20MR	8	40MR	16	8
40	70M	42	70MS	63	70MSS	63	80MSS	72	90S	90	60M	36	61
41	80MS	72	100S	100	100S	100	100S	100	100S	100	100S	100	95
42	80MS	72	100S	100	100S	100	100S	100	100s	100	100S	100	95
43	70MSS	63	90S	90	100S	100	100S	100	100S	100	100S	100	92
44	60MS	54	30MR	12	90MSS	81	80MSS	72	100S	100	80MSS	72	65
45	20R	8	50M	30	40M	24	30M	18	30M	18	40MR	16	19
46	50M	30	40M	24	80MSS	72	80MSS	72	90S	90	80MSS	72	60
47	50M	30	50M	30	80MSS	72	80MSS	72	90S	90	80MSS	72	61
48	50MR	20	70MS	63	80MSS	72	80MSS	72	100S	100	80MSS	72	67
49	100s	100	100s	100	100s	100	100s	100	100s	100	100s	100	100

DS = final disease Severity, RT = reaction type, CI = Coefficient of Infection, ACI = Average Coefficient of Infection MR = moderately resistant, M = moderately resistant to moderately susceptible, MS = moderately susceptible, MSS = moderately susceptible to susceptible, S = susceptible.

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Classification of wheat germplasm by slow rusting parameters

To identify genetic relatedness among studied wheat genotypes and the depiction of genetic diversity in resistance reaction, Ward's method of classification was implemented. Cluster analysis (Fig. 1) revealed three major groups considering measured attributes of slow rusting. Group C involved genotypes which having resistant reactions besides stability across studied years. In this research, genotypes with susceptible reactions in inspected locations and over years were located in group C accompanied by susceptible control (G45) (Fig. 1). Group B of cluster analysis included genotypes that having to fluctuate reactions over years and locations and other hand named as unstable genotypes.

Yellow rust pathogenicity agents and greenhouse assay

To comprehensively study the pathogenic effect of wheat yellow rust and to identify effective resistance genes, leaf samples infected with the disease were collected from four climatic regions including Mashhad, Sari, Zarghan, and Ardebil, and evaluated using a differential set of wheat yellow rust (Johanson *et al.*, 1972). Differential genotypes testing resulted in "6E142A +, Yr27", "74E150A +, YR27", "38E158A +, YR27" and "134E150A +, YR27" isolates which was belonged to Ardebil, Sari, Mashhad as well as Zarghan regions respectively (Table 3). Considering identified virulence/avirulence genes, wheat genotypes carrying *Yr2*, *Yr6*, *Yr7*, *Yr9*, *Yr18*, *Yr25*, *YrA* genes can infect by yellow rust agent meanwhile wheat genotypes conferring *Yr1*, *Yr4*, *Yr5*, *Yr10*, *Yr24*, *YrSU*, *YrCV*, and *YrSP* genes are secure against yellow rust disease in all regions. Testing of studied wheat genotypes against four inferred *Puccinia striiformis* isolates in a greenhouse state showed the magnitude of resistance variability (Table 1).

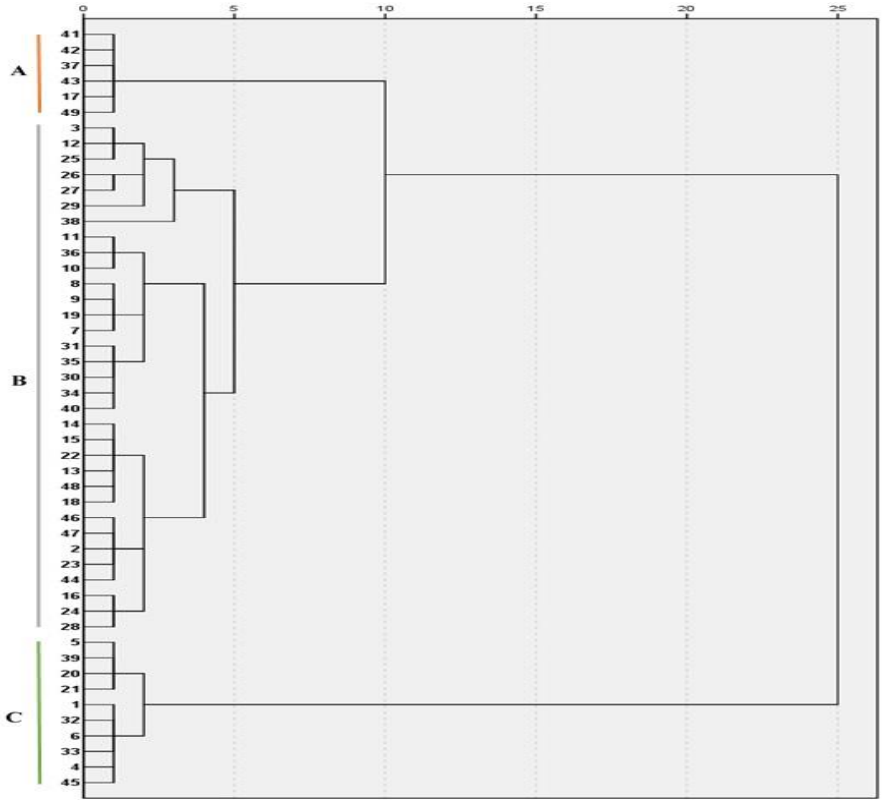

Figure 1 Ward classification algorithm distinguished wheat genotypes based on slow rusting parameters.

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Table 3 Races and Avirulence/ Virulence formula of yellow rust pathogen used in seedling test.

Race	Avir/vir Pattern	Location
38E158A +, YR27	***Yr1,Yr3,Yr4,Yr5,Yr10,Yr15,Yr24,Yr26,YrSU,YrCV,YrSP****/Yr2,Yr6,Yr7,Yr8,Yr9, Yr17,Yr18,Yr25,Yr27,Yr28, Yr29,Yr31,Yr32,YrSD,YrND,YrA*	Mashhad
6E142A +, Yr27	***Yr1,Yr3,Yr4,Yr5,Yr8,Yr10,Yr15,Yr24,YrSU,YrCV,YrSD, YrSP****/Yr2,Yr6,Yr7,Yr9, Yr17,Yr18,Yr25, Yr26,Yr27,Yr28,Yr29,Yr31,Yr32,YrND,YrA*	Ardebil
134E150A +, YR27	***Yr1,Yr3,Yr4,Yr5,Yr10,Yr15,Yr24,Yr26,Yr32,YrSU,YrCV,YrSD,YrND YrSP****/Yr2,Yr6,Yr7,Yr8,Yr9,Yr17,Yr18,Yr25,Yr27,Yr28,Yr29,Yr31,YrA*	Zarghan

In this regard, genotypes G1, G20, G21, G38, and G39 (Table 1) demonstrated similar resistant types and were resistant to 3 races and were sensitive to only one of the tested races. Identical data were obtained for G35, G36, G40, and G41 (Table 1) with the same types of reaction to infection by some pathotypes. This is highlighted that isolates "6E142A +, Yr27" and "38E158A +, YR27" out of identified isolates are the most aggressive and can be used in wheat future resistance breeding programs.

Discussion

Adult plant resistance is an important component of breeding wheat for resistance to stripe rust and several genetic analyses were done about it. A literature review (Boyd, 2006) manifested that resistance in field state is controlled by multiple genes which have additive effects. Like presented resistance will be more difficult for the pathogen to overcome than race-specific, single-gene resistance (McIntosh, 1995). Environmental factors can influence disease scores, in particular for a temperature-specific disease such as stripe rust (Pretorius *et al.*, 2007). In the present work, three geographical regions of Iran with different climatic characteristics were selected as experimental sites. Among regions, Ardebil has cold climate with the most cloudy weather and high humidity circumstance and therefore can be considered as rust compatible region compared to others such as Zarghan and Mashhad. Therefore, our findings showed that due to the existence of rigid pathogenic pressure in this region there was not seen any genotype with R reaction in Ardebil even over two years. Similarly, Hassan *et al.* (2022) proved that temperature has a negative correlation with the disease while humidity, rainfall, and wind speed have a positive correlation with disease development. Considering Table 2, just 10% of studied genotypes are MR type across the two years in the Ardebil region. Other studied regions Zarghan and Mashhad are located in southwest and northeast Iran with distinct circumstances that can influence the yellow rust development. Zarghan has warmer summers with low rainfalls compared with Mashahd. There was not any R type genotype in Mashhad albeit genotypes such as G04, G05, and G20 were MR type across two years. In the present work, the R type genotypes appeared in Zarghan region, and G05 out of the studied genotypes possessed R type reactions through two years in this region.

Cluster analysis as a multivariate statistical method is also a non-parametric stability method that can be implemented to identify resistant as well as stable genotypes. In this study, the classification of studied wheat germplasm using data of slow rusting attributes CI and DS across two years manifested three heterotic groups. Group C involved genotypes (G1, G04, G05, G06, G20, G21, G32, G33, G39, G45) that have the lowest average of CI as well as DS over years and locations. In contrast, group A possessed genotypes that were commonly susceptible over studied regions in years. Considering the method described by Ali *et al*. (2009) genotypes with CI values between 1-30%, 31-50% and 51-70% have high, intermediate, and low partial resistance against rust disease. Hence, group C contains highly partially resistant as well as stable genotypes. The identified heterotic groups can be used to select more distinct parental genotypes for wheat adult resistance breeding programs by identification of genomic regions controlling resistance reaction and new resistance genetic loci. Albeit seedling stage

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resistance is expressed throughout all growth stages (Feng *et al.*, 2018), it is mono-genetically inherited and follows the gene-for-gene concept (Flor, 1971) and can be broken down following pathogenic variability. In the present study, the introduced stable/resistant genotypes (group C) have more likely adult plant resistance genes for *P. striiformis* so they can be combined with seedling resistance to improve the general stripe rust resistance in commercial cultivars.

By using differential sets conferring several rust resistance genes, the genetic formula of each pathotype sampled from each region was recognized. Paralleled with previous reports (Afshari *et al.*, 2013) genes including Yr2, Yr6, Yr7, Yr9, and Yr25 were found as responsible for resistance against yellow rust in Iran. Screening of resistance reaction in greenhouse conditions depicted variation among studied genotypes in terms of yellow rust. In the greenhouse, some genotypes had similar resistance reactions against the four *P. striiformis* isolates which implies conferring the same resistance genes via these genotypes (Shynbolat and Aralbek, 2016). The resistance reaction in the seedling stage varied from that of find in the field state and some field-based resistant genotypes were susceptible in greenhouse conditions. For instance, field-based identified resistant genotypes G04, G05, G06, G20, G21, G32, and G33 were susceptible in the seedling stage which expressed the existence of adult plant resistance genes (Maccaferri *et al.*, 2015) in their genome.

Conclusion

Several resistant genotypes were identified in field conditions some of which probably carry new resistance genes. These findings showed the capacity of the presented wheat collection for genes and new sources of resistance to yellow rust. Multi-environmental yellow rust trials indicated temperature plays an important role in the pathogenicity of *P. striiformis*. Genotypes G1, G04, G05, G06, G20, G21, G32, G33, G39, and G45 were found resistant as well as stable ones regarding yellow rust disease. Compared with the greenhouse experiment, there appear to be adult plant resistance genes in field-based identified resistant genotypes that control resistance reactions.

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غربال گلخانه‌ای و مزرعه‌ای برخی از ژنوتیپ‌های گندم نان دیم کشور در برابر بیماری زنگ زرد

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چکیده: زنگ زرد گندم ناشی از عامل *Puccinia striiformis f.* sp. *tritici* یکی از مهم‌ترین بیماری‌های گندم است که تولید آن را تهدید می‌کند. مقاومت میزبانی اقتصادی‌ترین و سالم‌ترین روش مدیریت زنگ زرد است. در این مطالعه، پارامترهای مقاومت تدریجی به زنگ شامل ضریب آلودگی، شدت بیماری و هم‌چنین تیپ واکنش در ٤۸ ژنوتیپ گندم دیم به‌همراه تیمار کنترل حساس در شش محیط دیم یادداشتبرداری شد. آزمایش مزرعه‌ای طی دو سال متوالی در سه منطقه جغرافیایی مجزا شامل اردبیل، زرقان و مشهد انجام شد. هم‌چنین آزمایش غربال‌گری اضافی در شرایط گلخانه نیز انجام شد. نتایج نشان داد که از نظر مقاومت در برابر زنگ زرد تنوع ژنتیکی در ژرم پلاسم مورد مطالعه وجود دارد. در این تحقیق ژنوتیپ‌های G1، G04، G05، G06، G20، G21، G32، G33، G39، G45 از ژنوتیپ‌های مورد مطالعه در طول سال‌ها و مکان‌ها واکنش مقاوم و پایداری داشتند. با استفاده از الگوریتم خوشه‌بندی Ward سه گروه هتروتیک شناسایی شد که می‌توان از آن‌ها در برنامه‌های به‌نژادی زنگ زرد از طریق انتخاب والدین برای ایجاد جمعیت نقشه‌یابی این بیماری مهم گندم استفاده کرد. آزمایش واکنش ارقام افتراقی در مقابل جدایه‌های زنگ منجر به شناسایی جدایه‌های 6E142A+,Yr2 و 38E158A+,YR27 و 134E150A+,YR27 شد که به‌ترتیب مربوط به مناطق اردبیل، مشهد و هم‌چنین زرقان بودند. مقاومت در مرحله گیاهچه‌ای برای ژنوتیپ‌های مورد بررسی با واکنش آن‌ها در شرایط مزرعه متفاوت بود که نشان‌دهنده وجود ژن‌های مقاومت گیاه بالغ در ژنوم آن‌ها می‌باشد.

واژگان کلیدی: گندم، *Puccinia striiformis*، مقاومت گیاهچه‌ای، محیط دیم، مقاومت گیاه بالغ

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