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Research Article

Effective and ineffective resistance genes and reaction of promising wheat lines to stem rust in Ardabil

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Abstract: Stem (black) rust caused by *Puccinia graminis* f. sp. *tritici is* the most devastating of wheat diseases. Historically, it caused severe crop loss in many parts of the world. The cheapest and most environmentally friendly management strategy is the use of resistant wheat cultivars. Hence, the knowledge of effective resistance genes and determination of resistant sources will enable breeders to target those useful genes/resistant sources in their breeding programs. In order to determine effective resistance genes, virulence pattern of wheat stem rust was studied under the field conditions by planting of differential sets. Moreover, slow rusting parameters including final rust severity (FRS), apparent infection rate (*r*), relative area under disease progress curve (rAUDPC), and coefficient of infection (CI) were evaluated in a set of twenty-five wheat genotypes. The survey was conducted in Ardabil Agricultural Research Station, Northwest Iran, during two crop seasons 2013-2014 and 2015-2016. Results showed that there was no virulence for differential sets carrying resistance genes *Sr5, Sr13, Sr22, Sr24, Sr26 + Sr9G, Sr27, Sr32, Sr35 and Sr36*. But, virulence was observed for differential sets having resistance genes; *Sr25, Sr7a, Sr23*, *Sr28, Sr29, Sr30, Sr33, Sr34, Sr37, SrDP2, SrGT, SrWLD*, *SrH*. The genes found effective against stem rust under natural conditions may be deployed singly or in combinations with durable resistance genes to develop high yielding resistant wheat cultivars. Based on the results of evaluations for slow rusting parameters, seven lines together with susceptible check that had the highest values of FRS, CI, *r* and rAUDPC, were selected as susceptible lines. Six lines showed moderate or moderately susceptible reaction (M, MR, MS). Accordingly, these lines with low values of parameters are supposed to have gene (s) for varying degrees of slow rusting resistance. The remaining lines may have low level of slow rusting resistance that need further study to elucidate their nature of resistance.

Keywords: Wheat, stem rust, effective *Sr* genes, slow rusting resistance

Introduction

Wheat is one of the world's most important crops and a major staple food for many people in Central, West Asia and North Africa (CWANA), including Iran. About 5.7 million hectares are sown with wheat in Iran, with an annual production of 11.1 million tons (Anonymous, 2016). However, wheat productivity is threatened by abiotic and biotic stresses, including the wheat rusts. Stem rust caused by *Puccinia graminis* Pers f. sp. *tritici* Eriks. & E. Henn. (*Pgt*) is the major production constraint in

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most wheat growing areas of the world (Denbel *et al*., 2013), often causing yield loss up to 100% on susceptible cultivars (Park, 2007).

In breeding programs for manipulating of new cultivars, annual monitoring of pathogen is needed to detect new pathotypes which can overcome resistance genes (McIntosh and Brown, 1997). Production and use of resistant cultivars is best control method for cereal rusts. To determine virulence genetics, seedling resistance genes are studied in differential cultivars and new isogenic lines. Nature of seedling resistance genes and adult plant resistance genes is different. Because, the former can be detected at the seedling stage and remain effective throughout all stages of plant growth (Bux *et al*., 2012). In contrast, the adult plant resistance is not detectable at seedling stage; therefore, adult plant resistance genes should be investigated at adult plant stage under field conditions (Singh *et al*., 2011a).

Screening of varieties against stem rust is a regular activity due to the dynamic evolutionary nature of the pathogen. The rust pathogens evolve into new races quickly through mutation, migration, recombination and somatic hybridization (Singh *et al*., 2011b).Wheat rusts are airborne, therefore local races can migrate to other areas and quickly become regionally and often globally predominant. Thus, virulence has been reported for many *Sr* genes worldwide. However, virulence for some genes or gene combinations may still be absent regionally (Singh *et al*., 2015).

The first study on virulence factors of wheat rust was conducted using trap nursery by Zadoks (Zadoks, 1961). In order to study annual changes of races and virulence factors of wheat stem rust, national experiments have been carried out in different countries (Jin *et al*., 2007; Nazari *et al*., 2008; Afshari, 2012; Singh *et al*., 2015). In some of these studies, virulence factors of pathogen have been distinguished, also effective resistance genes have been recognized. For example, monitoring of virulence factors and effective genes against stem rust, especially after emerging of Ug99, revealed that resistance genes *Sr5, Sr6, Sr7a, Sr7b, Sr8a, Sr8b*, *Sr9a, Sr9b, Sr9d, Sr9e, Sr9f, Sr9g, Sr9h, Sr10, Sr11, Sr12*, *Sr16, Sr17*, *Sr18, Sr19, Sr20, Sr21, Sr23*, *Sr24, Sr30, Sr31, Sr34, Sr36, Sr38, Sr41, Sr49, Sr54, SrMcN* and *SrWld-1* are no longer effective (Singh *et al*., 2015) and cannot be used in breeding programs.

Detailed information on pathogen change and its virulence, and epidemiological factors on pathogen movements provide a basis for development of early warning system (Yahyaoui *et al*., 2002).

Based on the review of Singh *et al*. (2015) and McIntosh *et al*. (2017), many *Sr* genes that confer resistance to different races of stem rust have been identified. Several of the designated genes are qualitative and race specific (Jin *et al*., 2008; Singh *et al*., 2011b). Of these genes and alleles, at least 38 are effective or partially effective against the Ug99 race group including *Sr33*, introgressed from the wild relative *Aegilops tauschii* and *Sr35*, transferred from *Triticum monococcum* to bread wheat (Periyannan *et al*., 2013; Saintenac *et al*., 2013; Yu *et al*., 2014). A major risk associated with the use of such race-specific genes is the ability of pathogens to defeat the genes when they are deployed singly in wheat cultivars as has been demonstrated by the Ug99 defeating *Sr24*, *Sr36* (Jin *et al*., 2008, 2009) and *SrTmp* (Patpour *et al.,* 2016). Thus, efforts to identify and incorporate genes that confer stable resistance are important (McDonald and Linde, 2002). Slow rusting resistance is a kind of resistance that is both race non-specific and durable (Sawhney, 1995). Slow rusting resistance is often described as partial resistance or adult-plant resistance. Such slow rusting resistances are polygenic and decrease the infection efficiency and retard growth and development of the pathogen, especially in adult plants (Hei *et al.,* 2015; Saleem *et al.,* 2015; Singh *et al*., 2017). According to Yu *et al.* (2014), a total of five designated wheat stem rust resistance genes i.e. *Sr2, Sr55, Sr56, Sr57* and *Sr58 c*onfer quantitative adult-plant resistance. The effects of race non-specific genes are pronounced in the post seedling growth stages (Nzuve *et al*., 2012). These genes are also characterized by non-hypersensitive responses (Navabi *et al.*, 2004; Singh *et al*., 2009).

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Resistant wheat cultivars to rusts have been developed through the national wheat improvement research program and in collaboration with the CIMMYT in Iran. However, most of the cultivars do not possess durable resistance and have showed susceptible reaction to Ug99 race group after their introduction (Patpour *et al*., 2014). In most cases, the failures were due to new virulent pathotypes/races and deployment of the same R-gene (s) in wide array of wheat cultivars (Admassu *et al*., 2012). According to Singh *et al*. (2015), most stem rust resistance genes present in wheat cultivars and breeding lines of most countries as well as Iran are race specific and ineffective against most of the prevalent races of Ug99 group.

Considering the rapid evolution and spread of new virulent races of stem rust, the frequent failure of new cultivars with stem rust resistance and the limited availability of sources of durable resistance, it is imperative to develop new wheat cultivars using different sources of resistance. Therefore, the objective of this study was to determine the virulence patterns of the pathogen populations and effective resistance genes during two crop seasons 2013-2014 and 2015-2016, as well as identification of sources of adult plant, slow rusting resistance to stem rust in Iranian wheat lines.

Materials and Methods

This survey was subdivided into two experiments. First, virulence and avirulence factors were studied under field conditions during two cropping years, 2013-14 and 2015-2016. Also evaluation of slow rusting resistance parameters in a number of wheat promising lines from 2014 to 2016, in Ardabil province of Iran was made.

Determination of effective and ineffective resistance genes

46 differential sets and isogenic lines along with susceptible check (Morocco) used in this study are listed in Table 1. This experiment was carried out under natural conditions at Ardabil Agricultural Research Station (38°17' N, 48°39' E, elevation: 1380m) during two cropping years, 2013-14 and 2015-16. The differential sets received from SPII (Seed and Plant Improvement Institute) were used to identify virulence and avirulence against current populations of stem rust pathogen in present study. Each entry was planted in two 1 meter rows which were spaced 30cm apart. Plots were spaced at 65cm. A susceptible spreader (Morocco) was sown around the borders of the experiment and 10 entries intervals. All required cultural practices were carried out during the experiment. Disease severity was estimated according to the modified Cobb's scale; 0% = immune, and 100% = fully susceptible (Peterson *et al.,* 1948) when disease was well-developed at the flag leaf stage. The infection type (IT) of disease was also recorded based on Roelfs *et al.* (1992). The presence of virulence factors was determined by susceptible infection type while monitoring the disease on differential sets. In other words, corresponding genes against virulence factors of pathogen in plants (with severity and infection type more than 50S) were considered as ineffective genes and corresponding genes against avirulence factors of pathogen were considered as effective resistance genes (Bux *et al*., 2011; Safavi and Afshari, 2017b).

Study of slow rusting parameters

This experiment was conducted under natural infection condition at Ardabil Agricultural Research Station (Iran) during 2013-14 and 2015-16 cropping seasons. 24 promising wheat lines along with susceptible check used in this study are listed in Table 2. Each entry was planted in two rows of 1 meter spaced at 30cm apart. Plots were spaced at 65cm. Experimental design was randomized complete block design with three replications. Disease severity was recorded three times, starting when the susceptible check Morocco reached 40% severity using modified Cobb's scale (Peterson *et al.,* 1948) and infection type based on Roelfs *et al.,* (1992). Coefficient of infection (CI) was calculated by multiplying disease severity (DS) and constant values of infection type (IT). The constant values for infection types were used based on; R = 0.2, MR = 0.4, M = 0.6, MS = 0.8, S = 1 (Stubbs *et al.,* 1986).

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Table 1 Wheat genotypes used in trap nursery, their resistance genes, disease severity and infection types produced by stem rust during two years (2014 and 2016).

Genotypes	Gene/s[1]	Severity and infection type[2]	
		2014	2016
ISR5RA	*Sr5*	10MR	30MR
W2691SR6	*Sr6*	10S	50S
LINE G	*Sr7a*	10S	80S
ISR7BRA	*Sr7B*	20S	60S
ISR8ARA	*SrR8A*	20S	70S
BARLETA BENVENUTO	*Sr8B*	20S	60S
ISR9ARA	*Sr9A*	20MS	60S
W2691SR9B	*Sr9B*	20MS	60S
ISR9DRA	*Sr9D*	10MR	50MS
VERNSTEIN	*Sr9E*	10MR	40MS
ISR5SB	*Sr9F*	10S	50S
CNS(TC2B)/LINE E	*Sr9G*	20S	50MS
W2691SR10	*Sr10*	20MS	60S
ISR11RA	*Sr11*	20S	40MSS
CH.SP.(TC3B)	*Sr12*	10S	50S
W2691SR13	*Sr13*	20MS	30MR
LINE A SELN.	*Sr14*	30MS	50S
W2691SR15NK	*Sr15*	30S	60S
ISR16RA	*Sr16*	20S	80S
LC/KENYA HUNTER	*Sr17*	40S	50MSS
LCSR19MG	*Sr19*	40S	60S
LCSR20MG	*Sr20*	50S	70S
T.MONOCOCCUM DERIV	*Sr21*	30MS	50S
SWSR22T.B.	*Sr22*	20MSS	30MR
EXCHANGE	*Sr23*	30S	50MSS
BT SR24 A9	*Sr24*	10MR	30MR
LC SR25 ARS	*Sr25*	40MSS	70S
EAGLE	*Sr26 + Sr9G*	20MR	30MR
COORONG TRITICALE	*Sr27*	5R	R
W2691SR28KT	*Sr28*	30S	50MSS
PUSA/EDCH	*Sr29*	20MSS	70S
BTSR30WST	*Sr30*	40S	70S
LINE E/KVZ	*Sr31*	20S	50S
C77.19	*Sr32*	20MR	30M
TETRA CANTHATCH/AG.SQUARROSA(RL5045)	*Sr33*	20S	60S
COMPARE	*Sr34*	20MS	60S
W3763	*Sr35*	20MS	40M
W2691 SRTT1	*Sr36*	20MS	20MR
W2691 SRTT2	*Sr37*	40S	60S
FED.*2/SRTT3	*SrTT3 + Sr10*	80S	80S
MEDEA AP9D	*SrDP2*	5R	30M
BTSRGAMUT	*SrGT*	10MS	60S
PELISS	*SrPL*	10MR	50S
BT/WLD	*SrWLD*	20S	70S
H44 DERIV	*SrH*	30S	70S
Morocco	-	30S	100S

1: Resistance genes based on the studies of Singh *et al.* (2015) and Afshari (2012).

2: Infection types based on Roelfs *et al.* (1992); 0 = Immune. R = Resistant without sporulation. TMR = trace moderately resistant. MR = moderately resistant; small pustules surrounded by necrotic areas. MS = moderately susceptible; medium-sized pustules, no necrosis, but some chlorosis possible. MSS = moderately susceptible to susceptible; medium to large sized pustules without chlorosis or necrosis. S = susceptible; large pustules, no necrosis or chlorosis.

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Table 2 Pedigree of wheat lines**,** adult plant infection type, and mean values for coefficient of infection, final rust severity, infection rate and rAUDPC in 25 wheat lines to stem rust in Ardabil in two years 2014 and 2016.

Line code	Pedigree/Parents	Infection type	Mean values of slow rusting parameters[1]			
			FRS	CI	rAUDPC	*r*
MS-87-8	1-66-22/3/Alvd//Aldan/Las	MSS	63	57	44	0.141
DW-90-4	SOMAT_4/INTER_8/3/EUPODA_3/SLA_2//MINIMUS	MSS/S	80	76	68	0.167
DW-90-8	SOOTY_9/RASCON_37//STORLOM	MSS	73	66	44	0.141
DW-90-13	SORA/2*PLATA_12//SOMAT_3/4/STORLOM/3/RAS…	MSS	63	57	39	0.126
DM-88-17	NA[2]	MSS	67	60	41	0.13
S-89-15	SLVS*2/PASTOR	MSS	63	57	42	0.128
S-91-6	Alvand//Aldan"s"/IAS58/3 /Vee/Nac	MSS	67	60	42	0.138
S-91-13	PFAU/MILAN/5/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/VEE#7/BOW/4/PASTOR	MSS	63	57	42	0.134
S-91-15	PRL/2*PASTOR/4/CHOIX/STAR/3/HE1/3*CNO79//2*SERI	MSS	57	51	37	0.126
C-85-3	Ghk"S"/Bow"S"//90Zong87/3/Shiroodi	MSS	57	51	34	0.113
C-88-4	Gascogene/Col No.3625//Alamoot	MSS	57	51	41	0.134
C-91-4	Zrn/Shiroodi/6/Zrn/5/Omid/4/Bb/Kal//Ald/3/Y50E/Kal*3//Emu	MS	57	46	35	0.129
CD-91-8	Jagger 'sib'/3/Lagos-7//Guimatli 2/17	MSS	90	81	97	0.187
CD-91-11	Zander//Attila/3*Bcn (-0SE-0YC-0YE-3YE-0YE-2YE-0YE)	MS	53	42	40	0.143
CD-91-12	Solh	MSS	57	51	43	0.141
N-90-7	OASIS/SKAUZ//4*BCN/3/2*PASTOR	MSS	67	60	44	0.112
N-91-8	PFAU/MILAN/5/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/VEE#7/BOW/4/PASTOR	MR/M	30	15	22	0.082
N-91-9	PFAU/MILAN/3/SKAUZ/KS94U215//SKAUZ	MS	43	34	29	0.091
N-91-10	TILHI/5/PF74354//LD/ALD/4/2*BR12*2/3/JUP//PAR214*6/FB6631/6/ATTILA/2*PASTOR	M	37	22	23	0.085
N-91-17	MILAN/S87230//BABAX	M/MS	50	35	31	0.11
WS-89-7	Kauz/Pastor/PBW343	MR/M	37	19	22	0.095
WS-90-10	Falat/Barakat/ 5/Omid/4/ Bb/Kal//Ald/3/Y50E/3*Kal/Emu	MSS	63	57	39	0.138
WS-90-18	CROC_1/AE.SQUARROSA (2247)//OPATA/3/PASTOR	S	83	83	78	0.176
M-90-16	SHARP/3/PRL/SARA/TSI/VEE#5/5/VEE/LIRA//BOW/3/BCN/4/KAUZ	M	40	24	29	0.093
Check	Morocco	S	100	100	100	0.256

1: Abbreviations: Final rust severity (FRS), coefficient of infection (CI), relative area under disease progress curve (rAUDPC), apparent infection rate (*r*), moderate (M), moderately resistant (MR), moderately susceptible (MS), moderately susceptible to susceptible (MSS), susceptible (S).

Estimation of area under disease progress curve (AUDPC) and relative area under disease progress curve (rAUDPC) was performed as described by Milus and Line (1986).

Also the infection rate (*r*) was estimated in terms of disease severity recorded on wheat lines in different times (Van der Plank, 1968). The infection rate (*r*) per unit (*t*) was calculated as follows:

$$r = 1/t_2\text{-}t_1[(\ln(x_2/1\text{-}x_2))\text{-}(\ln(x_1/1\text{-}x_1))]$$

Where t_1 and t_2 are dates at which disease severity measurements were made, and x_1 and x_2 are the amounts of disease recorded on these dates. Finally comparison of lines was used for grouping of them based on the method of Ali *et al*. (2007) and Patahn and Park (2006). SPSS software (Version 18) was used for cluster

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analysis (using UPGMA method) and generating denderogram for grouping of promising lines.

Results and Discussion

Effective and ineffective resistance genes

The differential sets and isogenic lines showed wide range of rust response during two years' investigation (Table 1). The field data obtained in 2013-14 and 2015-16 cropping seasons revealed that genotypes having resistance genes *Sr5, Sr13, Sr22, Sr24, Sr26 + Sr9G, Sr27, Sr32, Sr35, Sr36* showed low level or no infections, and therefore, were effective (Table 1). The genotypes with resistance genes *Sr25, Sr7a, Sr23*, *Sr28, Sr29, Sr30, Sr33, Sr34, Sr37, SrDP2, SrGT, SrWLD*, *SrH* showed reactions more than 50S at least in one year and were considered as susceptible genotypes. Therefore, these genes were ineffective against race group of Ardabil population. Although, some resistance genes such as *Sr31, Sr6, Sr9F, Sr12, Sr14, Sr21* and *SrPL* showed reactions near to 50S at least during cropping season 2015-2016, they were not selected as effective or ineffective resistance genes under Ardabil conditions. Reaction of wheat lines during two years were different. The different reaction of some genotypes can be due to different weather conditions in the two years, 2014 and 2016 or, different races were predominant during 2014 and 2016. Therefore reactions of some genotypes did not confirm each other based on the field experiment. Planting date can also affect severity and infection types of stem rust in some places such as Ardabil. Therefore, for confirming the reactions of some genotypes, more experiments (at seedling and adult plant stages) need to be conducted.

Previously, Afshari (2012) reported that stem rust isolate of Dasht-Azadeghan (from Khuzestan province of Iran) didn't show virulence on plants carrying genes *Sr5, Sr22, Sr24, Sr26 + Sr9G, Sr27,* and *SrGT*. In study of Jin *et al*. (2007), resistance genes *Sr13, Sr22, Sr24, Sr25, Sr26, Sr27, Sr27, Sr28, Sr32, Sr33, Sr35, Sr36, Sr37, Sr39, Sr40, Sr44* and *SrTmp*had low reactions to race of TTKSK in seedling and in field nursery at Njoro. Different researchers around the world also reported that most of the race- specific resistance genes except for some of them, such as*Sr22, Sr26, Sr33, Sr35, Sr45* and *Sr50* (Jin *et al*., 2007; Nazari *et al*., 2008; Afshari, 2012; Singh *et al*., 2015) are ineffective resistance genes. In present study, resistance genes *Sr5, Sr13, Sr22, Sr24, Sr26 + Sr9G, Sr27, Sr32, Sr35 and Sr36* were effective. The study shows some differences between our results with those of other researches. For example in this study, virulence was not observed for resistance genes *Sr24* and *Sr36*, whereas, other researchers (Jin *et al*., 2008, 2009; Singh *et al*., 2015) showed that the genes *Sr24* and *Sr36* are no longer effective against some variants of Ug99. The differences between results may be due to variation of environmental conditions where the experiments were conducted or due to difference in race populations.

All of effective genes, used in this study, are race-specific (Singh *et al*., 2015) and should be deployed in combination with durable resistance genes such as slow rusting resistance genes. Building breeding programs around major genes poses vulnerability. In contrast, minor genes that were in combination with other (major and/or minor) genes provide durable resistance (Bux *et al*., 2011). Durable resistance for stem rust in many wheat varieties around the world has been attributed to the presence of *Sr2* (Singh, 1992) and other race-nonspecific resistance genes (Singh *et al*., 2011a, 2015). The race- nonspecific resistance genes *Sr2*, *Sr55, Sr56, Sr57* and *Sr58* in combination with each other (4-5 genes) have been proved to keep durability of resistance (Singh *et al*., 2011a). Some of these genes that are used in parents of new cultivars are present in the resistance germplasm sources such as Tukuru, Kukuna, Vivitsi, Kiritati, Parula, Pavon 76, Kingbird, Trap, Chapio, Toinchi 81, Attila and Muu (Singh *et al*., 2005, 2011a). Recent studies at CIMMYT have shown that gene *Lr46* is closely linked to genes *Yr29, Sr58* and *Pm39* (Singh *et al*., 2015). The gene*Yr46* is also closely linked to *Lr67* (Herrera-Foessel *et al*., 2011) and *Sr55* and *Pm46* (Singh *et al*., 2015). These genes confer slow rusting to yellow, leaf,

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stem rusts and powdery mildew. Another minor gene, *Yr30*, involved in adult plant resistance of several CIMMYT wheat lines was found to be at chromosomal region carrying durable stem rust resistance gene *Sr2* (Singh *et al.,* 2000). The genes *Yr29* and *Yr30* are widely distributed in CIMMYT wheat germplsm (Singh *et al*., 2005). Slow rusting gene *Sr57*is also closely linked to *Yr18, Lr34, Pm38, Sb1* and *Bdv1* (Singh *et al*., 2015). The *Lr34/Yr18* has a strong linkage with LTN (leaf tip necrosis) and also *Lr67/Yr46* is known to be associated with some degree of LTN (Rosewarne *et al*., 2006). LTN, a morphological trait, shows complete linkage or pleiotropism with *Yr18* and *Lr34* genes (Singh, 1992) and could be used as a marker to identify wheat lines carrying these genes (Shah *et al*., 2011) and select cultivars having durable resistance genes (Safavi and Afshari, 2012; Shah *et al*., 2010).

In recent years, new races of *P. graminis* f. sp. *tritici* (*Pgt*) have been reported in wheat production areas globally (Singh *et al*., 2008; Singh *et al*., 2015; Patpour *et al*., 2016). Wheat growing environments such as the east African highlands, with continual wheat production and favorable microclimates, are known hot spots for the rapid evolution and spread of new rust races. The occurrence and spread of virulent stem rust races in and out of the region has threatened wheat production globally (Periyannan *et al*., 2013).

Regarding the rapid changes of rust fungi (Singh *et al*., 2011b, 2015), therefore, it is recommended that selection for cultivar/line should be emphasized on multigenic resistance or partial resistance which is durable resistance. This kind of resistance can sustain yield production of wheat and prevent resistance break -down. If resistance gene sources were selected in combinations, we would have cultivars with different resistance genes which can delay occurrence of new virulent pathotypes. Considering the virulence factors of different races in different parts of Iran (Afshari, 2012; Nazari *et al*., 2013), breeding programs should be designed based on the results of this study and others on virulence factors. Some resistance genes are durable. These genes in combination with slow-rusting resistance genes such as *Sr2*, *Sr55, Sr56, Sr57*and *Sr58* from different resistant sources which have kept their resistance for a long time (Singh *et al*., 2015) should be deployed in order to produce durable resistant cultivars.

In this study, we found that *Sr5, Sr13, Sr22, Sr24, Sr26 + Sr9G, Sr27, Sr32, Sr35 and Sr36* were effective against the stem rust populations. Genes such as *Sr5, Sr13, Sr24, and Sr36* are previously known to show susceptible reaction to stem rust in different countries (Singh *et al*., 2015; Patpour *et al*., 2014; Nazari *et al*., 2013). So, we cannot use them in breeding programs. There are however other race-specific resistance genes such as *Sr22, Sr26, Sr33, Sr35, Sr45* and *Sr50* that still show resistance reaction to stem rust around the world (Singh *et al*., 2015) and, therefore can be used inbreeding program. These major genes along with others mentioned earlier have been found to confer resistance in differentials and or cultivars. Which of these genes (as single gene or in combinations) are present in the resistant Iranian cultivars however, remains to be studied.

Slow rusting parameters

The present study showed diversity in the final rust severity of the tested genotypes (Table 2) that may be due to differences in the number of resistance genes and mode of their action. Ali *et al*. (2009), Safavi and Afshari (2012) proposed that wheat lines with FRS values of 1-30%, 31-50% and 51-70% were considered as high, moderate and low levels of slow rusting resistance, respectively. The line N-91-8 was included in the first group, while lines N-91-9, N-91-10, N-91-17, WS-89-7 and M-90-16 exhibited moderate level of partial resistance and 13 other lines were identified to have low level of partial resistance.

Lines with a low FRS under high disease pressure may possess more additive genes (Singh *et al*., 2005) or genes with major effects. FRS represents the cumulative result of all resistance factors during the progress of epidemics. Many earlier researchers such as Ali

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et al. (2009), Shah *et al*. (2010), Tabassum *et al.* (2011), Safavi and Afshari (2012, 2017a) and Hei *et al.* (2015) also used final rust severity as a parameter to assess slow rusting behavior of wheat lines.

Previously Ali *et al*. (2009) considered that lines with CI values of 0-20, 21-40, 41-60 could possess high, moderate and low levels of slow rusting resistance, respectively. Based on the AUDPC values, Ali *et al*. (2009) categorized the wheat lines into two distinct groups. One group included lines exhibiting AUDPC value up to 30% of the check, and the second group included lines showing AUDPC value up to 70% of the check. The genotypes in group I were regarded as expressing good levels of slow rusting and that of group II were expressing moderate slow rusting resistance. According to Singh *et al*. (2005) wheat lines with variable field infection responses of MR-MS to MSS are expected to possess genes that confer partial resistance.

Infection rate in the present study showed more variation among the tested lines than disease severity and AUDPC, and it did not distinguish lines displaying different levels of slow rusting resistance with regard to other parameters. For example, line CD-91-11 has FRS, CI, and rAUDPC less than line N-90-7 but its infection rate is higher. Similar results were found for yellow rust, stem rust and leaf rust of wheat and barley (Ali *et al*., 2009; Safavi *et al*., 2013; Hei *et al*., 2015). Therefore, infection rate should be used in combination with other disease parameters.

Wheat line N-91-8 had high slow rusting resistance with low level of disease severity (1-30%), while lines N-91-9, N-91-10, N-91-17, WS-89-7 and M-90-16 had moderate levels of slow rusting resistance with FRS of 30–50% and CI values ranging from 21 to 40. According to Ali *et al*. (2009), genotypes in both groups I and II could have durable resistance, which can serve as good parents for breeding. Hence, lines in both groups are considered potentially useful in wheat breeding.

In this study some lines showed high or moderate levels of slow rusting resistance. Based on the pedigree analysis, the cultivar Pastor is present in the pedigree of some promising lines. This cultivar has *Sr2* slow rusting gene. This gene singly cannot provide adequate levels of resistance, but its combination with other slow rusting genes (4-5 genes) showed adequate levels of resistance (Singh *et al*., 2011a). Therefore, the lines having Pastor in pedigree with high or moderate levels of slow rusting most probably have other resistance gene or genes.

Diversity among the tested lines

Cluster analysis based on the slow rusting parameters is shown in Fig. 1. The Morocco cultivar along with three lines DW-90-4, CD-91-8, and WS-90-18 were separated with maximum distance from all the other lines which were grouped into two main clusters. So, the cluster C with high level of severity and infection type was not recommended for use in breeding programs. The first cluster (A) consisted of 15 lines six of which showed low level of slow rusting resistance and the other 9 were grouped as lines with very low level or without slow rusting resistance. The second cluster (B) comprised of six lines, characterized with the well-documented partially resistant lines N-91-8, N-91-9, N-91-10, N-91-17, WS-89-7 and M-90-16. Good parents, such as Pastor, Attila and Babax which were used in pedigree of some of the mentioned lines, carry at least two slow rusting resistance genes (Singh *et al*., 2005). Therefore, these lines most probably have different slow rusting resistance genes and be selected or involved in breeding programs. Diversity among tested lines was partially considerable in the disease parameters and cluster analysis based on slow rusting parameters to stem rust which in turn can be related to the diversity of the genetic basis of resistance among the tested lines. Other researchers (Ali *et al.* 2009; Hei *et al*., 2015) also reported varying degrees of partial resistance to wheat rusts among the commercial wheat cultivars/lines. The variation recorded in the present study may be exploited in breeding programs for developing improved genotypes with diverse resistance background. This will assist to prevent mono-culturing in terms of resistance genes.

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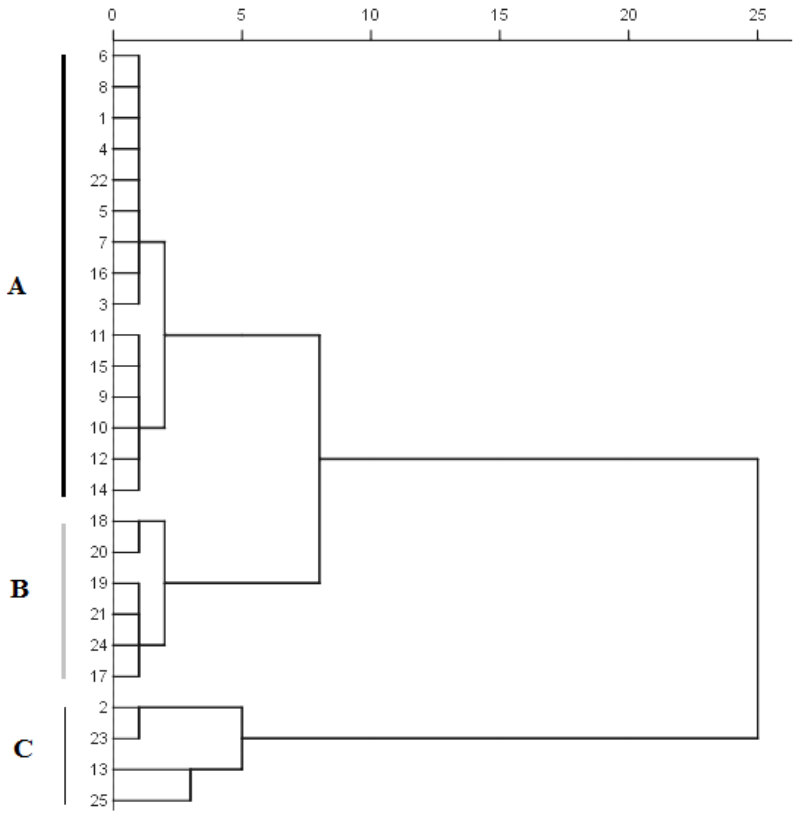

Figure 1 Denderogram of cluster analysis using UPGMA method for 25 wheat genotypes (numbers 1-24 for promising lines and 25 for susceptible cultivar) based on slow rusting parameters to stem rust.

Conclusion

Based on the results of this study and other researchers from Iran, genes*Sr22, Sr24, Sr26 + Sr9G, Sr27, Sr32, Sr35 and Sr36* are still effective in Ardabil and some parts of Iran and can be deployed in combination with durable resistance genes such as *Sr2, Sr55, Sr56, Sr57* and *Sr58* to develop new resistant wheat cultivars. In this study we also concluded that six lines showed moderate or moderately susceptible reaction (M, MR, or MS). Accordingly these lines with low values of parameters were supposed to have gene (s) for varying degrees of slow rusting resistance. The results of this study will assist in devising a strategy for stem rust management, using the well characterized wheat germplasms carrying effective resistance genes in the breeding programs in some parts of Iran that have virulence patterns or race populations like those in Ardabil province.

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ژن‌های مقاومت مؤثر و غیرمؤثر و واکنش لاین‌های امیدبخش گندم نسبت به زنگ سیاه گندم *Puccinia graminis* f. sp. *tritici* در اردبیل

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چکیده: زنگ سیاه گندم با عامل *Puccinia graminis* f. sp. *tritici* یکی از مخرب‌ترین بیماری‌های گندم است. از لحاظ تاریخی این بیماری خسارت‌های شدیدی را در بسیاری از نقاط جهان سبب شده است.ارزان‌ترین و از لحاظ محیطی سالم‌ترین روش مدیریت بیماری استفاده از ارقام مقاوم است. از این‌رو، دانش وآگاهی درخصوص ژن‌های مقاومت مؤثر و تعیین منابع مقاومت به‌نژادگران را قادر می‌سازد تا ژن‌های مفید یا منابع مقاومت را در برنامه‌های به‌نژادی استفاده کنند. بنابراین، این پژوهش در سال‌های ۱۳۹۴ و۱۳۹۶ به مدت دو سال به‌منظور تعیین کارائی ژن‌های مقاومت در اردبیل و شناسائی الگوی بیماری‌زایی عامل زنگ سیاه تحت شرایط مزرعه‌ای با کاشت ارقام افتراقی انجام شد. هم‌چنین در این پژوهش پارامترهای مقاومت تدریجی برای تعدادی از لاین‌های امیدبخش در شرایط مزرعه‌ای یادداشت‌برداری شد. نتایج بررسی نشان داد که ژن‌های مقاومت *Sr5*، *Sr13*، *Sr22*، *Sr24*، *Sr9G*، *Sr26*، *Sr27*، *Sr32*، *Sr35* و *Sr32* ژن‌های مقاومت مؤثری بودند و ژن‌های مقاومت *Sr25*، *Sr6*، *Sr9f*، *Sr7a*، *Sr23*، *Sr28*، *Sr29*، *Sr30*، *Sr33*، *Sr34*، *Sr37*، *SrDP2*، *SrGT*، *SrPL*، *SrWLD*، *SrH* ژن‌های غیرمؤثری در طی دوره پژوهش بودند. ژن‌های مقاومت مؤثر ممکن است به‌تنهایی یا در ترکیب با ژن‌های مقاومت پایدار (غیراختصاص-نژادی) جهت ایجاد ارقام با عملکرد بالا استفاده شوند. نتایج ارزیابی‌ها برای پارامترهای مقاومت تدریجی نشان داد که هفت لاین همراه با رقم حساس بالاترین مقادیر FRS، CI، *r* و rAUDPC را داشتند، و بنابراین به‌عنوان ارقام حساس گروه‌بندی شدند. تعداد کمی از لاین‌ها (شش لاین) در مرحله گیاه کامل واکنش متوسط (MR, M, MS) نشان دادند و سطح بالا یا متوسطی از مقاومت تدریجی را داشتند. بقیه لاین‌ها هم به دلیل دارا بودن مقادیر بالای پارامترهای اندازه‌گیری شده دارای سطح پایین مقاومت تدریجی بودند و برای اثبات طبیعت مقاومت آنها نیاز به مطالعه بیش‌تری است.

واژگان کلیدی: گندم، زنگ سیاه، ژن‌های مقاومت مؤثر، مقاومت تدریجی

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