Journal of Plant Physiology and Breeding

2023, 13(2): 113-130 ISSN: 2008-5168

Research paper

Biometrical analysis of resistance to stem rust (*Puccinia graminis* f. sp. tritici) in the winter wheat genotypes

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Received: May 7, 2022 Accepted: November 29, 2022
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Abstract
Stem rust or black rust is one of the most important fungal diseases that widely affect wheat yield and quality in the world. Therefore, the selection of genetic materials resistant to stem rust in the breeding programs is necessary. In this study, 24 winter wheat genotypes including eight varieties and 16 elite lines were evaluated at the adult plant and seedling stages using a randomized complete block design under the influence of local stem rust race TKTTF. Disease indices including the type of infection, the severity of infection, the coefficient of infection, the area under the disease progress curve (AUDPC), the relative area under the disease progress curve (rAUDPC), and genotype reaction were recorded. Significant differences were observed among the genotypes for all disease indices. Based on all indices, MV-17 and C-98-17 were resistant and C-98-14, C-98-9, Bolany, and Morocco were susceptible. Pearson's correlation coefficients revealed a significant positive correlation between field type of infection, the severity of infection, the coefficient of infection, AUDPC, and rAUDPC at the field, and between greenhouse type of infection and genotype reaction at the greenhouse. Based on cluster analysis by Ward's method, all the genotypes were classified into four groups (R, MR, MS, and S) in the adult plant stage and into two groups (R and S) at the seedling stage. The resistant genotypes can be used in the breeding programs for improvement of the stem-rust-resistant genotypes.

Keywords: cluster analysis, diversity, resistance, rust, wheat

How to cite: Vahed Rezaei A, Asghari A, Norouzi M, Aharizad S, Roohparvar R, Amini A. 2023. Biometrical analysis of resistance to stem rust (*Puccinia graminis* f. sp. tritici) in the winter wheat genotypes. J Plant Physiol Breed. 13(2): 113-130.

Introduction

Rusts are the most important pathogens of the wheat crop (Knott 2012). The main rusts are stem rust (*Puccinia graminis* f. sp. tritici)(Pgt), yellow/stripe rust (*Puccinia striiformis f. sp. tritici*), and leaf/brown rust (*Puccinia triticina*) (Poland and Rutkoski 2016), however, stem rust is more dangerous to wheat than the other two rusts (Park 2016). Stem rust is seen mainly in warm and moist conditions. Its typical symptoms are red-brick urediniospores on the

leaf sheaths, glumes, awns, and stems (Kolmer 2005). This pathogen reduces the photosynthetic area, disrupts water and nutrient transport, and causes lodging, kernel shriveling, and yield loss in wheat plants (Knott 2012). This pathogen has several physiologic races (Roelfs 1985) such as TTKSK, TKTTF, TRTTF, JRCQC, TTTTF (Newcomb *et al.* 2016), and Ug99 (a virulent race detected in Uganda and Kenya (Wanyera *et al.* 2006). Since then, seven other races have been reported: PTKST, TTKSF, TTKSP, TTKST, TTTSK, TTKSF+, and PTKSK (Pretorius *et al.* 2012).

Rusts have been controlled efficiently by the use of genetic resistance (Olivera *et al.* 2015). However, the R-gene-type resistance has not been stable (Johnson 1983) because of the development of new races mainly due to sexual and para-sexual recombination (Burdon 1993), migration of the virulent variants into new areas (Singh *et al.* 2011), and climatic changes (Semenov and Halford 2009). Minor-genes-controlled partial resistance is viewed as durable compared to the resistance governed by the R-genes. However, both resistance types are complementary to each other in developing durable resistance (Hundie *et al.* 2018). Breeding for genetic resistance is considered the most economical and environmentally friendly method to combat the rust pathogen (Zhang *et al.* 2017). Many attempts have been made to achieve rust resistance in wheat and other cereals since genetic resistance can provide effective and chemical-free disease control (Mapuranga *et al.* 2022). Achieving this goal is possible only by having sufficient knowledge about the genetics of pathogen populations and identifying effective resistance genes in wheat genotypes (Roelfs *et al.* 1992).

Stem rust resistance can be found in the seedling and adult plant stages of wheat (Ellis *et al.* 2014). The number of stem-rust-resistance Sr genes has been cataloged and lines with unique Sr genes are available in several wheat backgrounds (Rouse *et al.* 2011). The results of recent research about the identification of new resistance sources in response to new races have shown that only Sr2, Sr13, Sr14, Sr22, Sr26, Sr28, Sr33, Sr35, Sr42, and Sr45 genes had effective resistance to stem rust races. Also, 12 new genes (Sr46 to Sr57) and several other genes have been identified as new sources of resistance (Jin *et al.* 2007; Hiebert *et al.* 2010; Singh *et al.* 2011; Ghazvini *et al.* 2012). Hiebert *et al.* (2016) indicated that the resistance in adapted germplasm is governed by relatively few resistance genes such as Sr2, Sr25, Sr26, SrCad, SrTmp, and Sr1A.1R. Also, Ug99 variants of the stem rust races such as PTKST, TTKSF, TTKSK, TTKSP, TTKST, and TTTSK with virulence to Sr21, Sr24, and Sr36 genes indicate that the race Ug99 is evolving on wheat (Jin *et al.* 2008; Jin *et al.* 2009).

This study aimed to evaluate the resistance of the 24 winter wheat genotypes to the stem rust pathogen in the field (adult plants) and greenhouse (seedlings) conditions and identify possible stem-rust-resistant genotypes in the East Azarbaijan Province, Iran.

Materials and Methods

Plant material

The seeds of the studied genotypes were provided by the Department of Cereal Research, Seed and Plant Improvement Institute, Karaj, Iran. The genotypes included eight cultivars from the Iran and Hungary winter wheat collection and 16 elite lines. The characteristics, origin, released year, and pedigree of the genotypes are presented in Table 1. Two cultivars, MV-17 and Morocco, were selected based on their distinctiveness in responses to stem rust. MV-17 was resistant (Dadrezaei *et al.* 2015) and Morocco was susceptible to stem rust (Denbel *et al.* 2013; Salcedo *et al.* 2017).

Pathogen, collection, and reproduction

An isolate of Pgt, identified as the race TKTTF, was used for evaluating the winter wheat genotypes. The isolate of TKTTF was collocated according to Woldeab *et al.* (2017). The infected stems and leaves were cut and labeled from wheat fields of Tabriz (37º 56′ 59.57″ N, 46º 03′ 49.10″ E), East Azarbaijan, Iran, in 2020 (Figure 1). The collected urediniospores after recovery in the Department of Cereal Pathology Lab, Seed and Plant Improvement Institute, were suspended

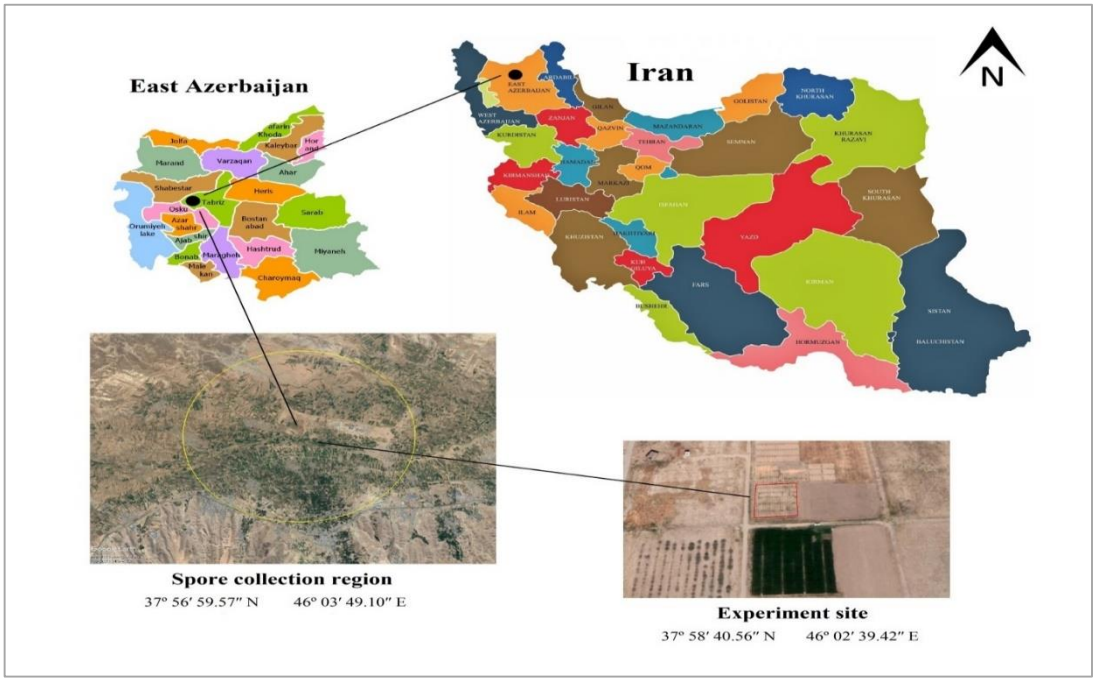

Figure 1. Pathogen collection area and adult plant evaluation site.

Table 1. Characteristics and pedigree of 24 wheat genotypes that was used in this study.

No.	Name	Cultivar	Origin	Release year	Pedigree
1	C-98-1	Mihan	Iran	2010	Bkt/90-Zhong 87
2	C-98-2	Haydari	Iran	2015	Ghk"s"/Bow"s"//90Zhong87/3/Shiroodi
3	C-98-3	Zarrineh	Iran	2017	Omid/4/Bb/Kal//Ald/3/Y50E/Kal*3//Emu"s"/5/Zrn/6/Zrn/Shiroodi
4	C-98-4	Zareh	Iran	2010	130L1,11//F35,70/Mo73/4/Ymh/Tob//Mcd/3/Lira
5	C-98-5	-	-	-	Alvd/4/Ghk"s"/Bow"s"//90Zhong87/3/Shiroodi
6	C-98-6	-	-	-	Alvd/4/Ghk"s"/Bow"s"//90Zhong87/3/Shiroodi
7	C-98-7	-	-	-	Charger//CMH80A.768/3*Cno79/3/Zrn
8	C-98-8	-	-	-	Charger//CMH80A.768/3*Cno79/3/Zrn
9	C-98-9	-	-	-	Spb"s"//K1349/Go/3/Vee"s"/4/Bkt/90-Zhong 87
10	C-98-10	-	-	-	Shahpasand/Norman
11	C-98-11	-	-	-	Alvd/4/Ghk"s"/Bow"s"//90Zhong87/3/Shiroodi
12	C-98-12	-	-	-	Alvd/4/Ghk"s"/Bow"s"//90Zhong87/3/Shiroodi
13	C-98-13	-	-	-	Alvd/4/Ghk"s"/Bow"s"//90Zhong87/3/Shiroodi
14	C-98-14	-	-	-	Spb"s"//K1349/Go/3/Vee"s"/4/Pishgam
15	C-98-15	-	-	-	AU/3/MINN//HK/38MA/4/YMH/ERA/5/PMF//CNO/GLL/6/KAUZ//ALTAR 84/AOS/7/TAM105/3/NE70654/BBY//BOW"S"/4/Century*3/TA2450
16	C-98-16	-	-	-	GRK79/TUKURU
17	C-98-17	-	-	-	MV NEMERE
18	C-98-18	-	-	-	ARS97135-9/O3A-B4//KS06O3A~49
19	CD-94-9	-	-	-	Zarrin/Shiroodi/6/Zarrin/5/Omid/4/Bb/Kal//Ald/3/Y50E/Kal*3//Emu
20	CD-94-5	-	-	-	Ga961565-27-6/La95283Ca-78-1-2
21	MV-17	MV-17	Hungary	1993	SLAVIA/MV-FT//BARANJK
22	CD-92-6	Heyran	Iran	2019	Lufer-1/Kinaci97
23	Morocco	Morocco	-	-	Susceptible check
24	Bolany	Bolany	-	-	Susceptible check

in lightweight mineral oil (Soltrol 170) and inoculated onto the fully expanded primary leaves of the seedling of McNair wheat cultivars. The inoculated seedlings were incubated in a dew chamber for 16 hours in the dark, and four hours under light. The inoculated seedlings were placed in a growth chamber at 7-12 ºC for three days and in the greenhouse at 18-25 ºC for two weeks. After infection of the seedlings, the race was multiplied and stored in the refrigerator at -80 ºC to use for screening of the genotypes at seedling and adult plant stages.

Adult plant evaluation

Twenty-four winter wheat genotypes were screened and evaluated for their level of infection to the stem rust pathogen in the field. The experiment was arranged as a randomized complete block design with three replications in the research field (37º 58′ 40.56″ N, 46º 02′ 39.42″ E; 1385m above sea level) of the East Azarbaijan Research Center for Agriculture and Natural Resources, East Azarbaijan, Iran, during 2019-2020 cropping season. Each experimental plot was 7.5 m^2 (5 m long and 1.5 m wide), with six rows, spaced 20 cm apart. The space between plots and blocks was 0.5 and 1.5 m, respectively. The information about the climate and temperature of the experimental site are presented in Figures 2 and 3. In general, the experimental site is

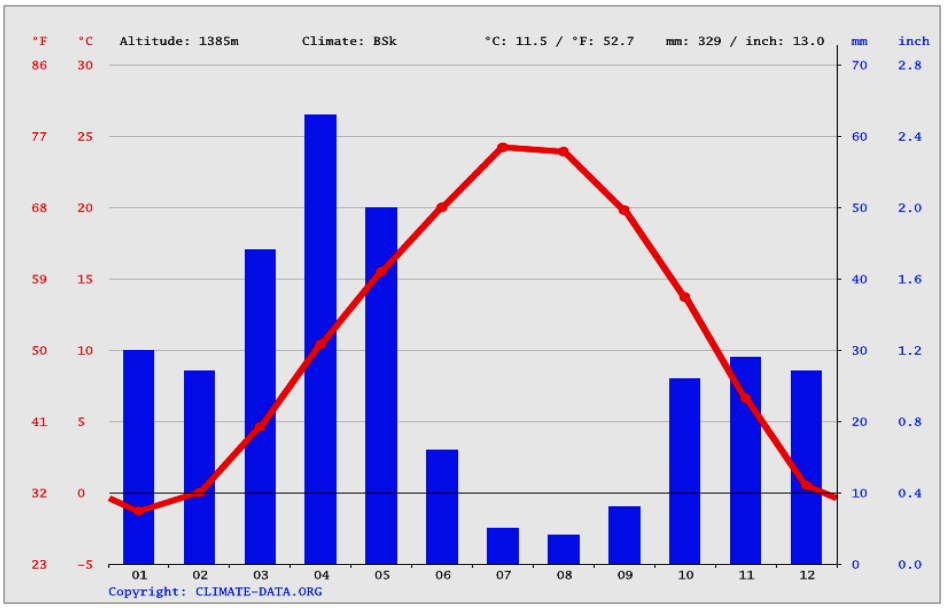

Figure 2. Monthly weather graph of the experimental site.

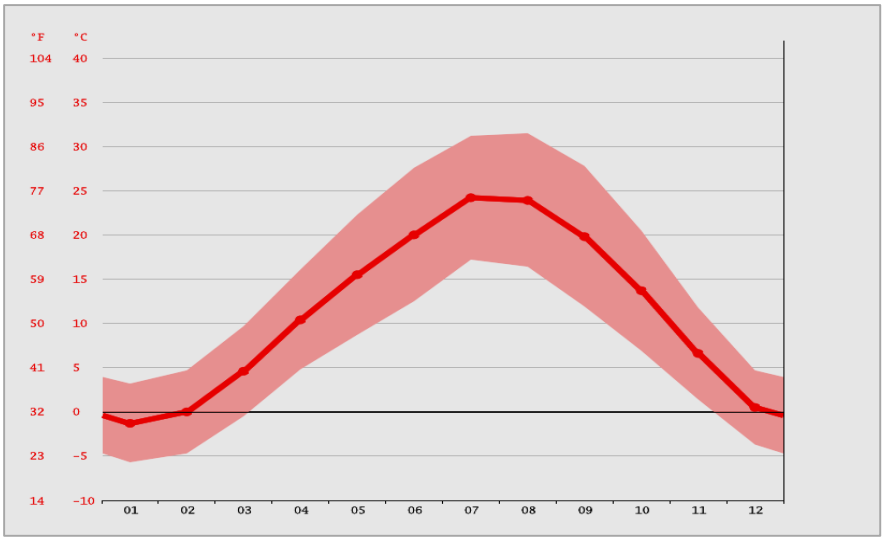

Figure 3. Average temperature of the experimental site.

characterized by local steppe climate, corresponding to BSk in the Köppen and Geiger classification, with two distinct seasons: a hot season from March to September and a cold season from November to February. The mean annual temperature is 11.5 ºC. The warmest month of the year is July with an average temperature of 24.2 ºC and January has the lowest average temperature of -1.3 ºC. The annual rainfall is 329 mm (Schwarz 2020). The physicochemical properties of the soil of the experiment are presented in Table 2. The site represents a proper condition for the stem-rust disease development. To inoculate the genotypes, all plots were inoculated with the Pgt race TKTTF

between the late booting and early heading stages. Plot surfaces were first sprayed with the deionized water and lightweight mineral oil (Soltrol 170) by 1 drop per liter after sunset, and then the stored spores at -80 ºC were mixed with talc powder (5:1 ratio) and sprayed onto the plants by a spore gun. The disease was scored on 10 plants from each plot. Disease scoring started a week after the spraying and continued three times at 5-day intervals (Roelfs *et al.* 1992) (Figure 4, Table 3). Also, the stem rust infection severity was estimated based on Peterson *et al*. (1948) shown in Figure 5. The coefficient of infection was calculated by multiplying the disease severity by the field infection scores (Table 3). In the adult plant stage, area under the disease progress curve (AUDPC) and relative area under the disease progress curve (rAUDPC) were calculated by using the following formula (Wilcoxson *et al.* 1975), where x_i = injury intensity in the ith observation, and t = time at the ith observation:

$$\mathrm{AUDPC} = \sum_{i}^{n}\left[\left(\frac{x_i + x_{i+1}}{2}\right) \times (t_{i+1} - t_i)\right]$$

$$\mathrm{rAUDPC} = \left(\frac{\mathrm{AUDPC\ of\ each\ genotype} \times 100}{\mathrm{AUDPC\ of\ the\ susceptible\ genotype}}\right)$$

Table 2. Physico-chemical properties of the soil in the experiment.

Index	Unit	Value	Index	Unit	Value
Sand (> 0.02 mm)	%	60	pH	-	7.7
Silt (0.02-0.002 mm)	%	20	Total nitrogen	%	0.079
Clay (<0.002 mm)	%	20	Available phosphorous	$mg.kg^{-1}$	19
Depth	cm	0-30	Available potassium	$mg.kg^{-1}$	310
TNV^{+}	%	11	Fe	$mg.kg^{-1}$	8.6
Soil texture	-	Loamy sand	Zn	$mg.kg^{-1}$	0.86
Organic matter	$g\ kg^{-1}$	0.86	Cu	$mg.kg^{-1}$	1.1

[+]Total neutralizing value

Table 3. Description of the stem rust infection types and symptoms at the adult plant stage of wheat.

Infection types	Symptoms	Value
O	No visible infection on the plant.	0
R (Resistant)	Visible chlorosis or necrosis, no uredia are present.	0.2
MR (Moderately resistant)	Small uredia are present and surrounded by either chlorates or necrotic areas.	0.4
M (Intermediate)	Variable-sized uredia are present, some with chlorosis, necrosis, or both.	0.6
MS (Moderately susceptible)	Medium-sized uredia are present and possibly, surrounded by chlorotic areas.	0.8
S (Susceptible)	Large uredia are present, generally with little or no chlorosis and no necrosis.	1

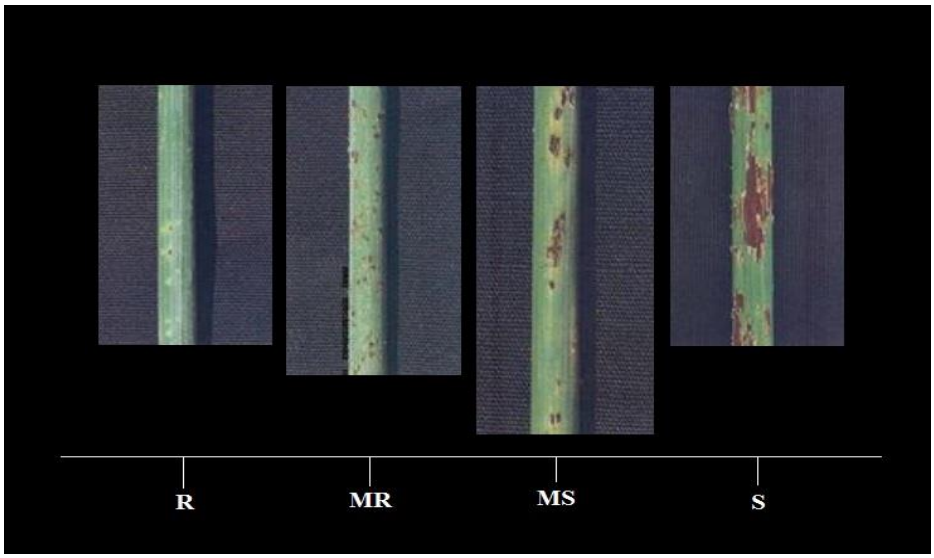

Figure 4. Adult plant infection type scoring scale recommended by Roelfs *et al.* (1992).

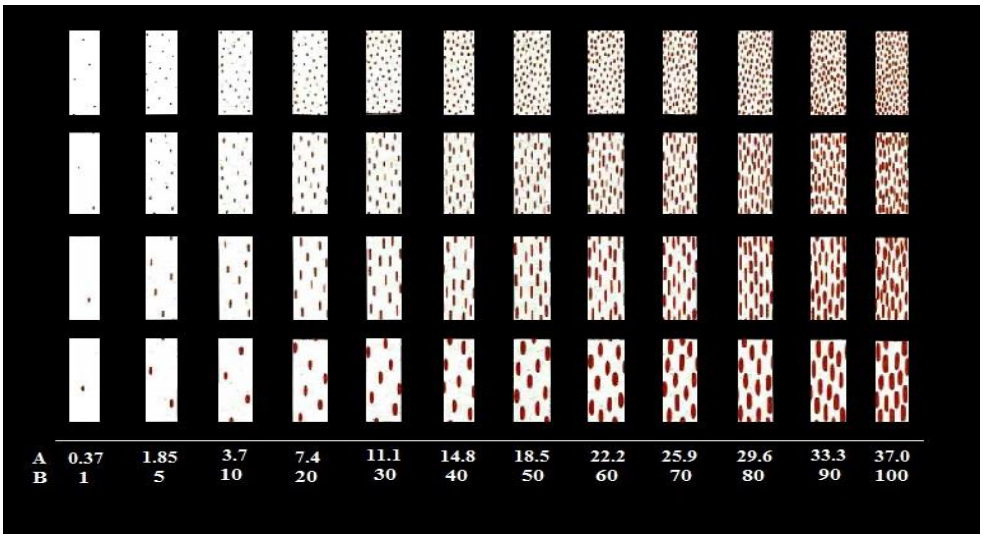

Figure 5. The adult plant infection severity scoring scale recommended by Peterson *et al.* (1948)

Seedling evaluation

For the seedling assessment, a randomized complete block design with four replications was conducted in a greenhouse in the Department of Cereal Research, Seed and Plant Improvement Institute, Karaj, Iran. Twenty seeds from each genotype were planted in a separate sterilized plastic pot with a diameter of 6 cm and a depth of 8 cm, filled with sterilized soil and peat moss with 1 to 1 ratio at 18 ºC. Seven days after germination, for infection of the seedlings, all pots were inoculated with the Pgt race TKTTF uredospores which were suspended with the light-weight mineral oil (Soltrol 170) by 50:50 % ratio and placed in a dew chamber for 24

hours of dark at 18 to 22 ºC. Then, the seedlings were returned to the greenhouse and kept at 21-24 ºC for 14 days (Woldeab *et al.* 2017). The infection type was assessed 14 days after inoculation using a modified 0-4 scale (Figure 6) according to Stakman *et al.* (1962).

Observations recorded in the field and greenhouse experiments were as follows: field type of infection (FTI), severity of infection (SI), coefficient of infection (CI), AUDPC, rAUDPC, genotype reaction at field (FGR), greenhouse type of infection (GTI), and genotype reaction at greenhouse (GGR).

Statistical analysis

All statistical analyses including analysis of variance, comparison of means, calculating of correlation coefficients, cluster analysis, and discriminant function analysis were run using SPSS 23 and STATISTICA 12 software. Means were compared by Duncan's multiple range tests at the 0.01 significance level. Relationships between field and greenhouse traits were determined by Pearson's correlation coefficients (Benesty *et al.* 2009). Cluster analysis for grouping of the genotypes was carried out using Ward's method with squared Euclidian distance. The cutting point in the dendrogram was determined by the discriminant analysis.

Results

Adult plant stage Results of the analysis of variance for different disease indices at the adult plant and seedling stages are presented in Tables 4 and 5. Significant differences ($p \leq 0.01$) were observed among the studied genotypes for all indices under the stem rust infection conditions at the adult plant stage.

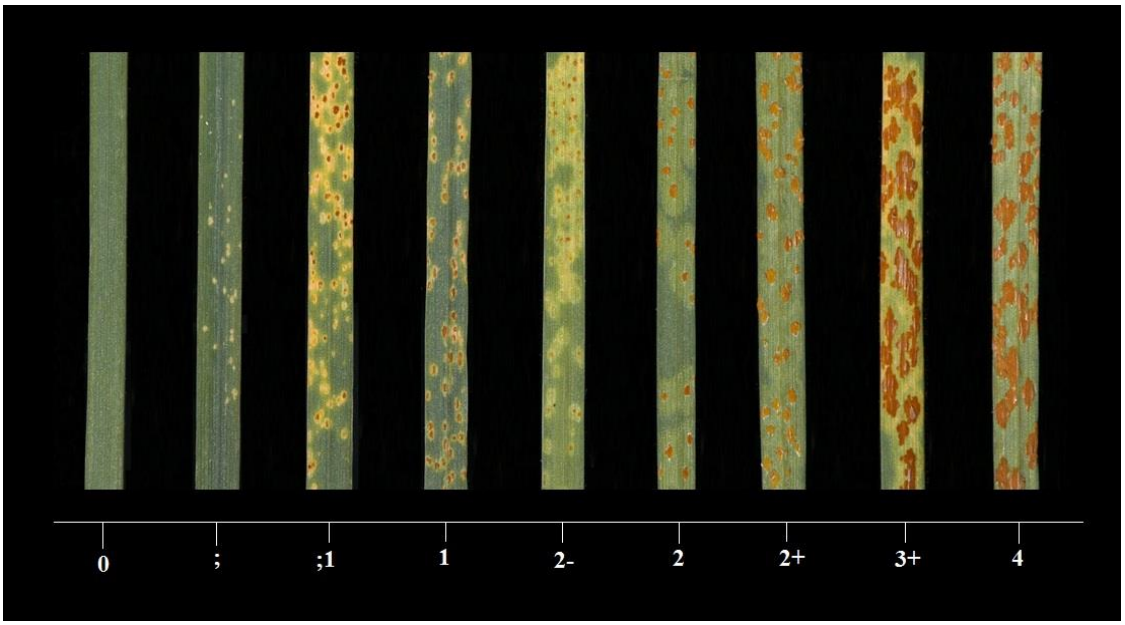

Figure 6. Seedling infection type scoring scale recommended by Stakman *et al.* (1962).

Table 4. Analysis of variance for different disease indices obtained from reaction to stem rust in the studied wheat genotypes at the field experiment.

SOV	df	Mean squares					
		FTI	SI	CI	AUDPC	rAUDPC	FGR
Replication	2	0.042	173.39*	137.81*	525.56*	286.54*	0.10
Genotype	23	0.210**	1707.48**	1718.08**	2626.91**	1432.26**	0.57**
Error	46	0.018	50.20	35.69	123.76	67.48	0.10
CV (%)		20.55	30.76	31.70	25.40	25.40	20.02

FTI: Field type of infection, SI: Severity of infection, CI: Coefficient of infection, AUDPC: Area under the disease progress curve, rAUDPC: Relative area under the disease progress curve, FGR: Genotype reaction at field.
*, **: Significant at 5% and 1% levels of probability, respectively.

Table 5. Analysis of variance for the greenhouse type of infection (GTI) and genotype reaction at the greenhouse (GGR) obtained from the reaction to stem rust in the studied wheat genotypes at the greenhouse experiment.

SOV	df	Mean squares	
		GTI	GGR
Replication	3	0.028	0.066
Genotype	23	14.080**	0.739**
Error	69	0.521	0.044
CV (%)		8.70	12.35

**: Significant at 1% level of probability.

The disease indices of the 24 winter wheat genotypes at the adult plant stages are shown in Table 6. Based on the FTI index, two cultivars and 10 lines showed adult plant resistance, and six cultivars and six lines were susceptible. At the adult plant stage, 20% of the genotypes were susceptible, 30% were moderately susceptible, 16% were intermediate, 25% were moderately resistant, and 8% were resistant. MV-17 and C-98-17 were resistant, C-98-11, C-98-12, C-98-13, C-98-15, C-98-16, and C-98-18 were moderately resistant, C-98-5, C-98-7, and C-98-10 were intermediate, Haydari, Zareh, Heyran, C-98-6, C-98-8, CD-94-5, and CD-94-9 were moderately susceptible, and Mihan, Morocco, Bolany, C-98-9, and C-98-14 were susceptible genotypes. Among the 24 genotypes, Morocco, Bolany, Heyran, Haydari, Mihan, CD-94-5, CD-94-9, and showed the highest severity of infection (SI > 30), while MV-17 and C-98-17 showed the lowest disease severity (SI < 5) at the adult plant stage. MV-17 and C-98-17 also had the lowest CI, AUDPC, and rAUDPC while Morocco, Bolany, Heyran, Mihan showed the highest values of CI, AUDPC, and rAUDPC. In total, MV-17 and C-98-17 had a better resistance based on all disease indices as compared to other genotypes. On the other

hand, Morocco, Bolany, Heyran, Haydari, Mihan, CD-94-5, and CD-94-9 were susceptible to stem rust at the adult plant stage.

Pearson's correlation coefficients (Table 7) indicated a significant positive correlation among FTI, SI, CI, AUDPC, and rAUDPC. FTI and CI were highly correlated with AUDPC and rAUDPC. The correlations of CI with SI, AUDPC, and rAUDPC were higher than the correlation between FTI and CI.

The result of the cluster analysis for the wheat genotypes, based on different disease indices at the adult plant stage, is demonstrated in Figure 7. The genotypes were grouped into four major clusters, each comprising 10, 7, 5, and 2 genotypes, respectively. Group means and their percentage deviation from the grand mean for disease indices are shown in Figure 8. The genotypes with minimum values of FTI, SI, CI, AUDPC, and rAUDPC were grouped in Clusters 4 and 3, and the genotypes with maximum values were grouped in the

Table 6. Scores of reaction to stem rust for the studied wheat genotypes at the field and greenhouse conditions.

	Field exp.						Greenhouse exp.	
Genotype	FTI	SI	CI	AUDPC	rAUDPC	FGR	GTI	GGR
Mihan	S	30.00^{ef}	30.00^{d-f}	63.51^{h}	46.89^{h}	L	4	L
Haydari	MS	40.00^{gh}	27.33^{de}	60.60^{gh}	44.74^{gh}	L	3	L
Zarrineh	M	8.67^{a-d}	4.13^{a}	27.90^{a-d}	20.60^{a-d}	L	4	L
Zareh	MS	21.67^{de}	19.67^{cd}	51.00^{e-h}	37.66^{e-h}	H	4	L
C-98-5	M	13.33^{a-d}	10.00^{a-c}	27.23^{a-d}	20.11^{a-d}	L	2-	H
C-98-6	MS	6.67^{a-c}	5.00^{a}	28.27^{a-d}	20.88^{a-d}	L	4	L
C-98-7	M	11.67^{a-d}	8.67^{a-c}	24.00^{a-d}	17.72^{a-d}	H	3+	L
C-98-8	MS	11.67^{a-d}	9.33^{a-c}	38.19^{c-f}	28.2^{c-f}	H	3+	L
C-98-9	S	10.00^{a-d}	10.00^{a-c}	43.66^{d-h}	32.24^{d-h}	L	4	L
C-98-10	M	16.67^{cd}	10.67^{a-c}	40.12^{c-g}	29.62^{c-g}	L	2-	H
C-98-11	MR	7.00^{a-c}	2.73^{a}	15.34^{ab}	11.33^{ab}	L	2	H
C-98-12	MR	6.67^{a-c}	2.67^{a}	20.88^{a-c}	15.42^{a-c}	H	3	H
C-98-13	MR	16.67^{cd}	6.67^{ab}	32.51^{a-e}	24.01^{a-e}	L	2	H
C-98-14	S	16.67^{cd}	16.67^{bc}	50.15^{e-h}	37.03^{e-h}	L	3	L
C-98-15	MR	15.00^{b-d}	6.00^{ab}	35.83^{b-e}	26.45^{b-e}	H	2C	H
C-98-16	MR	8.33^{a-d}	3.33^{a}	20.72^{a-c}	15.3^{a-c}	H	3+	L
C-98-17	R	2.33^{ab}	0.47^{a}	11.94^{a}	8.81^{a}	H	1+	H
C-98-18	MR	5.33^{a-c}	2.07^{a}	20.68^{a-c}	15.27^{a-c}	L	4	L
CD-94-9	MS	50.00^{h}	40.00^{f}	60.35^{gh}	44.56^{gh}	L	4	L
CD-94-5	MS	36.67^{fg}	29.33^{de}	58.39^{f-h}	43.11^{f-h}	L	4	L
MV-17	R	1.00^{a}	0.20^{a}	12.13^{a}	8.96^{a}	H	2-	H
Heyran	MS	46.67^{gh}	37.33^{ef}	62.33^{ef}	46.02^{ef}	L	3+	L
Morocco	S	100.00^{j}	100.00^{h}	135.43^{j}	100.00^{j}	L	4	L
Bolany	S	70.00^{i}	70.00^{g}	109.63^{i}	80.95^{i}	L	4	L

FTI, SI, CI, AUDPC, rAUDPC, FGR, GTI, GGR, L and H is: field type of infection, severity of infection, coefficient of infection, area under the disease progress curve, relative area under the disease progress curve, field genotype reaction, greenhouse type of infection, greenhouse genotype reaction, low and high, respectively.

Table 7. Pearson's correlation coefficients of different disease indices obtained from reaction to stem rust in the studied wheat genotypes at the field and greenhouse experiments.

	FTI	SI	CI	AUDPC	rAUDPC	GIT	FGR	GGR
FTI	1							
SI	0.62^{**}	1						
CI	0.65^{**}	0.99^{**}	1					
AUDPC	0.75^{**}	0.96^{**}	0.98^{**}	1				
rAUDPC	0.75^{**}	0.96^{**}	0.98^{**}	1^{**}	1			
GTI	0.65^{**}	0.43^{**}	0.45^{**}	0.50^{**}	0.50^{**}	1		
FGR	0.32^{ns}	0.33^{ns}	0.32^{ns}	0.33^{ns}	0.32^{ns}	0.11^{ns}	1	
GGR	0.63^{**}	0.36^{ns}	0.39^{ns}	0.44^{**}	0.44^{**}	0.95^{**}	0.08^{ns}	1

FTI, SI, CI, AUDPC, rAUDPC, FGR, GTI, GGR, L, and H are field type of infection, the severity of infection, coefficient of infection, the area under the disease progress curve, the relative area under the disease progress curve, field genotype reaction, greenhouse type of infection, greenhouse genotype reaction, low and high, respectively.

Clusters 1 and 2. Cluster 1 had susceptible genotypes and Cluster 4 had resistant genotypes, while Clusters 2 and 3 had moderate susceptible and moderate resistant reactions in response to the stem rust.

Seedling stage

The results of the analysis of variance for different disease indices are presented in Table 5 for the greenhouse experiment. Significant differences ($p \leq 0.01$) were observed among the genotypes for both GTI and GGR at the seedling stage.

The disease indices of 24 winter wheat genotypes at the seedling stage are presented in Table 6. Based on the GTI index, one cultivar and six lines showed seedling resistance and seven cultivars and 10 lines were susceptible at the seedling stage. In terms of the seedling reaction, 66% of the genotypes were susceptible and 34% were resistant. At the seedling stage, MV-17 and C-98-17 were resistant, C-98-11, C-98-13, and C-98-15 were moderately resistant, C-98-5 and C-98-10 were intermediate, C-98-8, Haydari, and Heyran were moderately susceptible, and Mihan, Morocco, Bolany, C-98-9, and C-98-14 were susceptible. C-98-12, C-98-16, C-98-18, CD-94-9, and CD-98-5 didn't show similar reactions at the adult plant and seedling stages.

Pearson's correlation coefficients indicated a strong positive correlation between GTI and GGR (Table 7). There was no significant correlation between FGR and GGR at the seedling stage. Also, FGR at the seedling stage was not significantly correlated with any of the disease indices at the adult plant stage. However, GGR at the seedling stage was moderately and significantly correlated with FTI, AUDPC, and rAUDPC, and highly and significantly correlated with GIT at the adult plant stage.

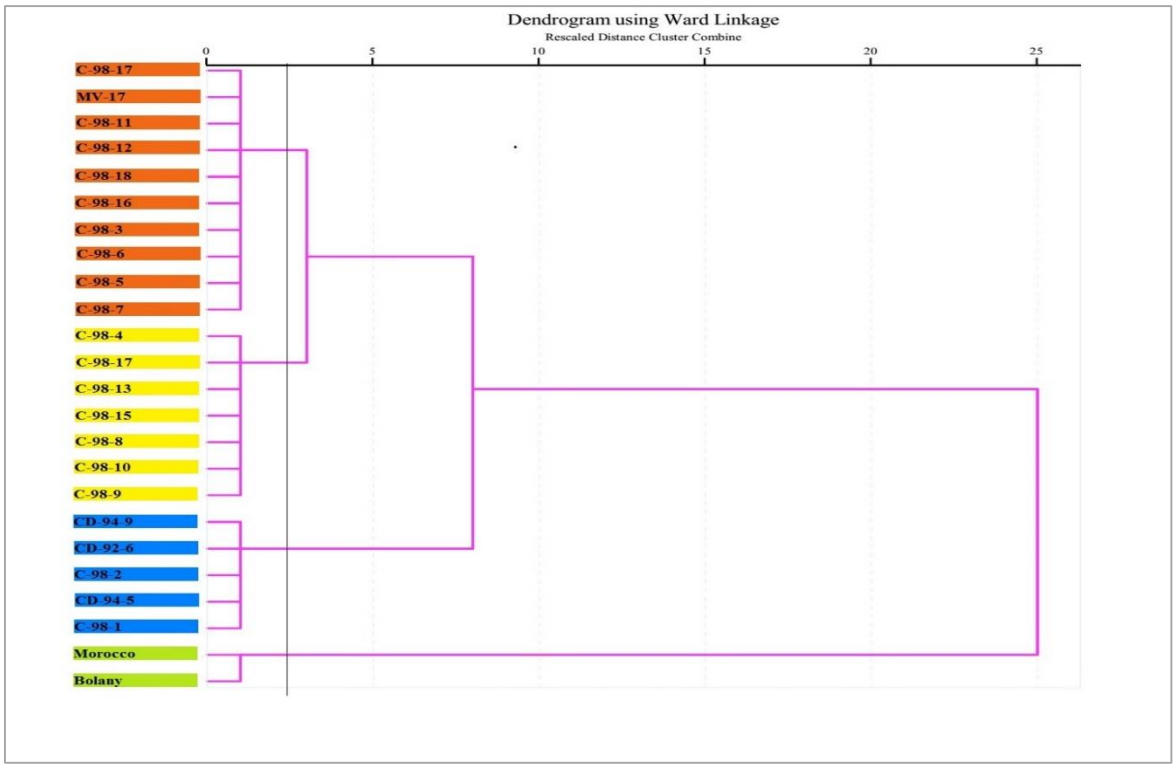

Figure 7. Dendogram of cluster analysis based on disease indices obtained from reaction to stem rust in the studied wheat genotypes by Ward's method at the field experiment. Orange = Cluster 4, Yellow = Cluster 3, Blue = Cluster 2, and Green = Cluster 1.

The result of the cluster analysis for the wheat genotypes based on different disease indices is shown in Figure 9 at the seedling stage. The genotypes were grouped into two clusters, which comprised 17 and 7 genotypes, respectively. Also, based on the mean of groups and their percentage deviation from the grand mean (Figure 10) at the seedling stage, genotypes with minimum and maximum values of GTI were grouped in Clusters 2 and Cluster 1, respectively. In other words, at the seedling stage, Cluster 1 had susceptible genotypes and Cluster 2 had resistant genotypes.

Discussion

Stem rust races are responsible for up to 100% yield loss of wheat. Therefore, breeders evaluate the resistance and genetic diversity of wheat genotypes to control this disease (Knott 2012). The existence of significant variability among the genotypes provides an opportunity for improving stem rust resistance in breeding programs. In this respect, our results were similar to the reports of Degete and Chala (2019), Taye *et al.* (2013), Hundie *et al.* (2018), and Soresa (2018). In this study, AUDPC was between 8.81 to 135.43 which was due to the expression of different resistance genes at the adult plant stage. The

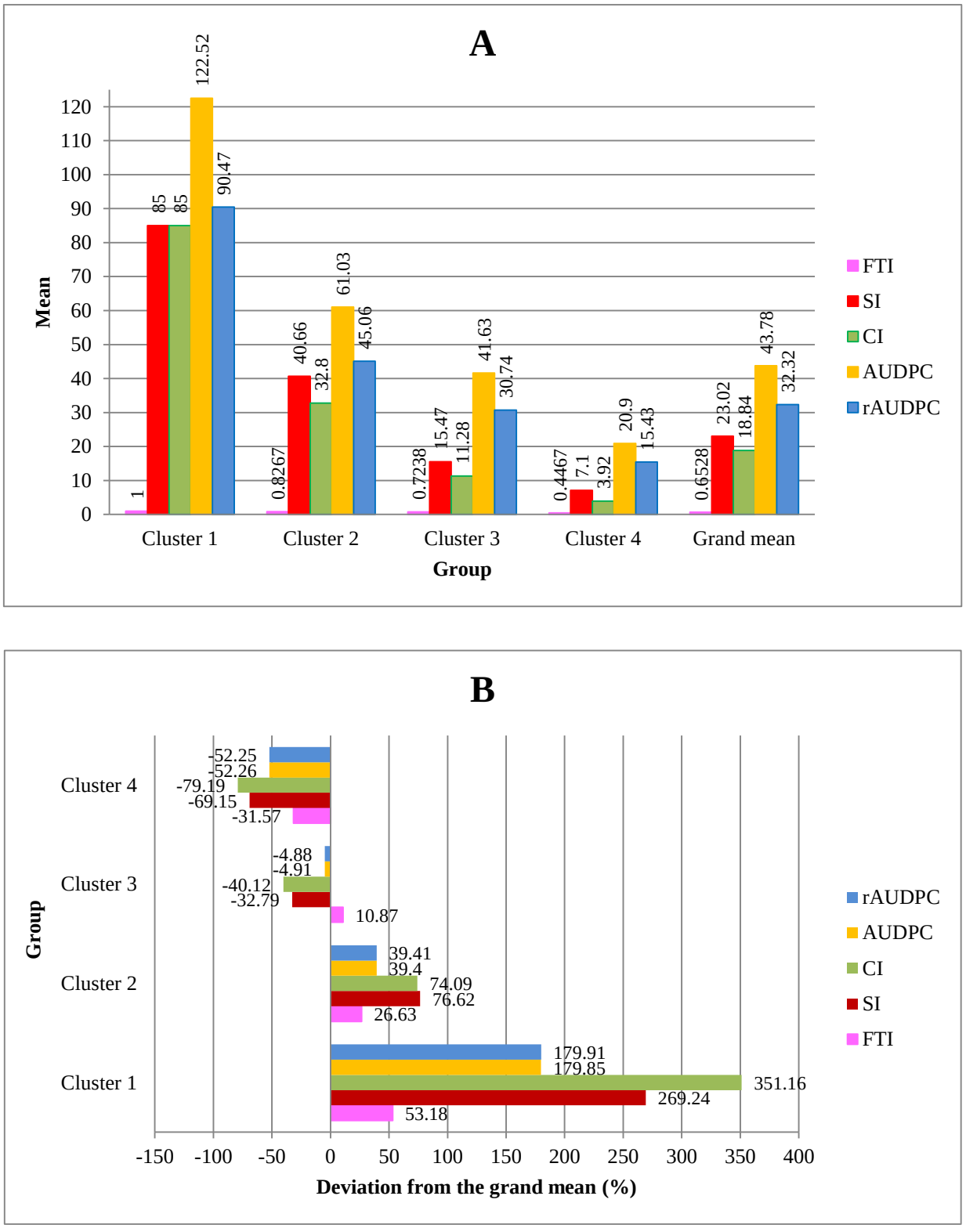

Figure 8. Group means (A) and their percentage deviation from the grand mean (B) based on the disease indices obtained from reactions to stem rust in the studied wheat genotypes at the adult plant stage.

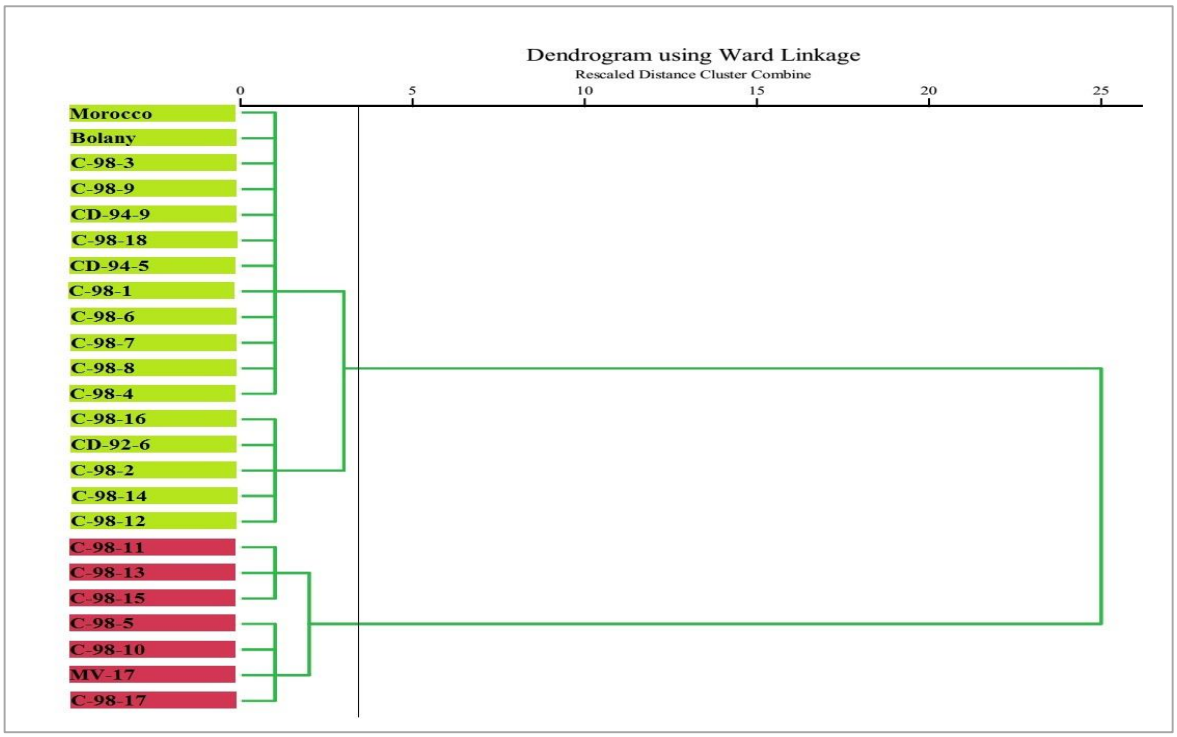

Figure 9. Dendogram of the cluster analysis based on disease indices obtained from reactions to stem rust in the studied wheat genotypes at the seedling stage (Green = Cluster 1) and (Red = Cluster 2).

genotypes that showed some resistance at the seedling and adult plant stages, such as MV-17, C-98-17, C-98-11, and C-98-5, may contain seedling resistance Sr gene that controls resistance reaction at the seedling stage or they may have minor genes that are working together to reduce the disease. However, the genotypes that showed some resistance only at the adult plant stage, such as C-98-12, C-98-18, C-98-16, C-98-3, C-98-6, C-98-5, and C-98-7 may only have adult plant resistance Sr gene expressed at this stage (Roelfs 1992).

The results of the present study showed that the stem rust resistance level of C-98-17 was comparable to the MV-17 check variety. Also, the resistance of lines C-98-11, C-98-12, C-98-18, and C-98-16 was close to MV-17. Thus, these plant materials could have resistance genes in their background and other unknown resistance genes (Hundie *et al.* 2018) and can be used in wheat breeding programs to produce rust-resistant varieties. At the seedling stage, C-98-17, C-98-10, and C-98-5 displayed almost comparable indices to MV-17. Also, based on disease indices, C-98-17 and C-98-9 were resistant and susceptible elite lines at both adult plant and seedling stages, respectively, which were derived from MV NEMERE and Spb"s"//K1349/Go/3/Vee"s"/4/Bkt/90-Zhong 87 pedigree. C-98-17 seems a promising resistant line at both adult plant and seedling stages.

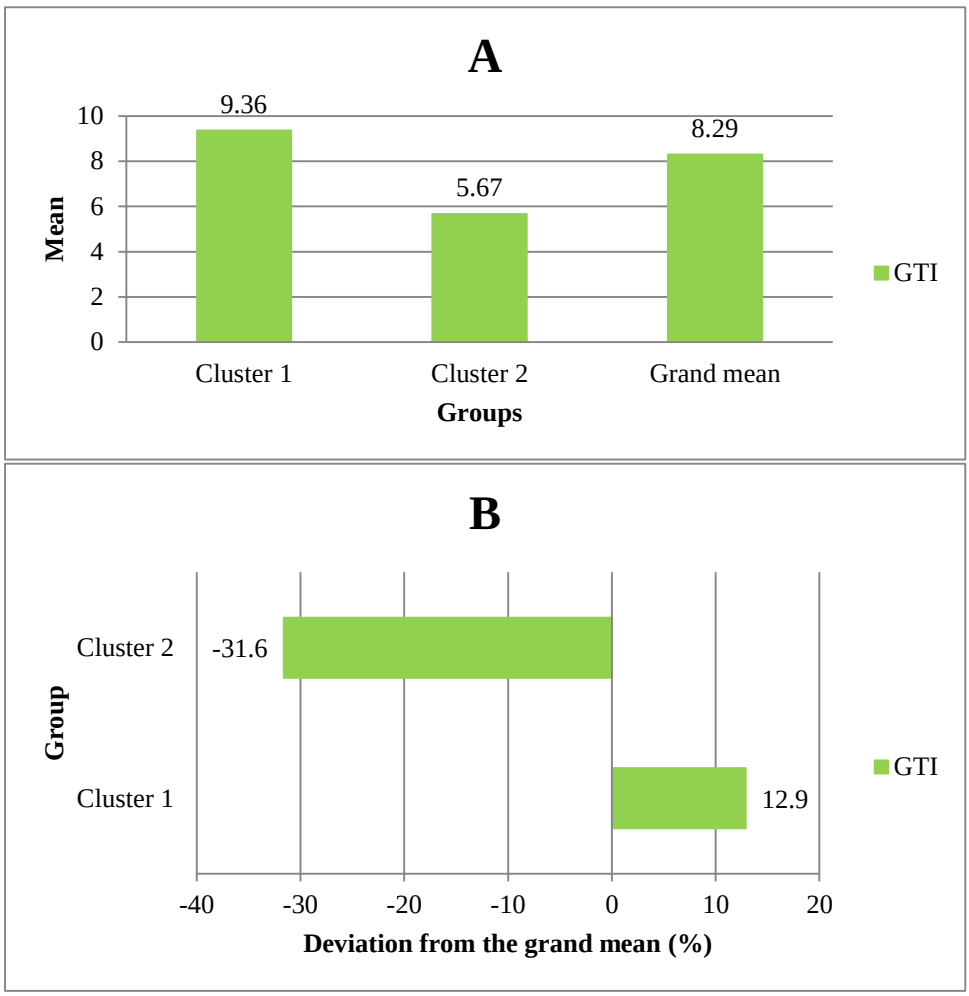

Figure 10. Mean of groups (A) and their percentage deviation from the grand mean (B) based on disease indices obtained from reactions to stem rust in the studied wheat genotypes at the seedling stage.

Conclusion

This study demonstrated the infection and pathogenicity of East Azarbaijan stem rust race TKTTF toward the winter wheat cultivars and elite lines at the field (adult plant stage) and greenhouse (seedling stage) conditions. This race was effective in both experimental conditions. At both adult plant and seedling stages, C-98-17 had resistance to stem rust very similar to MV-17 check cultivar. This line, together with several moderately resistant lines (such as C-98-11, C-98-12, C-98-18, and C-98-16), can be in the breeding programs to improve stem- rust resistance. The cluster analysis showed the existence of adequate genetic diversity for the studied stem rust disease indices, which will be useful in the rust-resistance breeding of winter wheat genotypes. However, the variability of environmental conditions influences the response of genotypes to pathogens. Therefore, this study should be repeated in

different locations and years.

Acknowledgments

Thanks to Seed and Plant Improvement Institute and East Azerbaijan Research Center for Agriculture and Natural Resources that supported this project. Sincere gratitude goes to Seed and Plant Improvement Institute, Cereal Research Department for providing the wheat genotypes, trial sites, and technical assistance. Final gratitude goes to Ms. Zohreh Bayat for their assistance in providing experimental sites and technical assistance to this project.

Conflict of Interest

The authors declare that they have no conflict of interest with any people or organization concerning the subject of this manuscript.

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بررسی بیومتریکی مقاومت به زنگ ساقه (*Puccinia graminis* f. sp. tritici) در ژنوتیپ‌های گندم پاییزه

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چکیده

زنگ ساقه یا زنگ سیاه یکی از مهم‌ترین بیماری‌های قارچی می‌باشد که به‌طور گسترده عملکرد و کیفیت گندم مناطق مختلف جهان را تحت تاثیر قرار می‌دهد. بنابراین گزینش منابع ژنتیکی مقاوم به زنگ ساقه در برنامه‌های به‌نژادی ضروری می‌باشد. در این پژوهش ۲۴ ژنوتیپ گندم شامل هشت رقم و ۱۶ لاین امید بخش در دو مرحله گیاهچه‌ای و گیاه بالغ در قالب طرح بلوک‌های کامل تصادفی و تحت تاثیر نژاد بومی TKTTF مورد ارزیابی قرار گرفت. شاخص‌های بیماری شامل نوع آلودگی، شدت آلودگی، ضریب آلودگی، سطح زیر منحنی پیشرفت بیماری (AUDPC)، مقدار نسبی سطح زیر منحنی پیشرفت بیماری (rAUDPC) و واکنش ژنوتیپ مورد یادداشت برداری قرار گرفت. تفاوت معنی‌داری بین ژنوتیپ‌ها از نظر تمامی شاخص‌های بیماری مشاهده شد. براساس تمامی شاخص‌های مورد مطالعه MV-17 و C-98-17 به عنوان ژنوتیپ‌های مقاوم و C-98-14، C-98-9، بولانی و مراکش به عنوان ژنوتیپ‌های حساس شناسایی شدند. همبستگی معنی‌دار بین نوع آلودگی، شدت آلودگی، ضریب آلودگی، AUDPC و rAUDPC در مزرعه و نیز بین نوع آلودگی و واکنش ژنوتیپ در گلخانه در پاسخ به زنگ ساقه وجود داشت. براساس تجزیه خوشه‌ای به روش Ward کلیه ژنوتیپ‌ها در مرحله گیاه بالغ به چهار گروه (مقاوم، نیمه مقاوم، نیمه حساس و حساس) و در مرحله گیاهچه به دو گروه (مقاوم و حساس) طبقه بندی شدند. بنابراین استفاده از ژنوتیپ‌های مقاوم این مطالعه در برنامه‌های اصلاحی برای ایجاد مقاومت به زنگ ساقه توصیه می‌شود.

واژه‌های کلیدی: تجزیه خوشه‌ای، تنوع، زنگ، گندم، مقاومت