Bulgarian Journal of Agricultural Science, 29 (No 6) 2023, 1057–1064

Evaluation of the resistance to *P. triticina* of Bulgarian common and durum wheat cultivars under controlled conditions and in infection field

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Abstract

Ivanova, V. & Boyadzhieva, S. (2023). Evaluation of the resistance to *P. triticina* of Bulgarian common and durum wheat cultivars under controlled conditions and in infection field. *Bulg. J. Agric. Sci., 29*(6), 1057–1064

During 2018 – 2020, in the infection field of Dobrudzha Agricultural Institute – General Toshevo, Bulgaria, 34 common winter wheat and 5 durum wheat cultivars were tested for resistance to leaf rust (*P. triticina*). The response of the common wheat cultivars to the *P. triticina* pathotypes predominant in this period was studied under controlled conditions, at seedling stage. The full set of pathotypes from the pathogen population identified during the respective year of study was taken out to the infection field to achieve a maximum rate of infection accumulation on the standard susceptible cultivar *Michigan amber*. The field parameters final disease severity (FSD), average coefficient of infection (ACI) and resistance rate were estimated. The type of resistance of the studied cultivars was determined. The results showed that at seedling stage most of the cultivars had compatible, susceptible reaction to the *P. triticina* pathotypes used in the study. Under the conditions of the infection field, 7.7 % of the cultivars exhibited high level of resistance (VR). Cultivar Zhana responded with stable resistance (R), and the cultivars with this type of resistance constituted 2.6%. Moderate resistance (MR) was registered in 5.1% of the tested cultivars. With very resistant to resistant reaction (VR – R) responded 18% of the cultivars. Resistant to moderately resistant (R – MR) was the reaction of 43.6% of the investigated cultivars, and 12.8% reacted within the range of high to moderate resistance (VR – MR). Some cultivars demonstrated variation of resistance within a wider range. The cultivars, which were within the range of highly resistant to moderately susceptible (VR – MS) constituted 5.1%, as well as those within the range moderately resistant to moderately susceptible (MR-MS). In some cultivars, such as Merilin, the level of resistance remained the same as in the previous years, regardless of the variations in the pathogen population, while in other cultivars, such as Karina, Enola and Lazarka, the resistance varied over years. All cultivars with stable resistance under the conditions of the infection field (from high to moderate), can be used in the breeding programs as parental components for the development of varieties resistant to leaf rust.

Keywords: Puccinia triticina; wheat; cultivars; resistance; types of resistance; adult plant resistance

Introduction

Wheat is attacked by numerous diseases, which decrease the yield and quality. Leaf rust is among the most common and harmful diseases and poses a real and constant threat to the sustainable production of wheat (Rolfs et al., 1992; Huerta-Espino et al., 2011).

The development of the disease primarily reduces the assimilation surface, which may cause changes in such parameters as water exchange, intensity of transpiration, and leaf water retention capability (Leushkina, 2008). In young plants affected by the disease, the formation of roots and stems is retarded and the plants are less resistant to the unfavorable climatic factors as a result. The heavy damages affect the bread making properties of the grain not allowing the formation of the low-molecular weight gluten components. All this influences the amount and quality of the yield (Stepanov, 1975).

The resistance of the plants to diseases has been subject to investigations for many years now. There are different concepts and theories trying to classify and explain different manifestations and mechanisms of the resistance. In this respect, the pathosystem wheat – *P. triticina* is among the most suitable for fundamental and specific studies (Todorova & Andonova, 2011).

The incorporation of resistance genes in the commercial cultivars is considered the cheapest and the most environmentally friendly method for control of the disease (Ittu, 2000). In modern agriculture, wheat breeding for resistance to *P. triticina* is mainly based on the hypersensitive race-specific resistance of the *Lr* genes. This usually gives effective results in the control of the disease, but the pathogen populations can rapidly overcome the resistance of the genes with the occurrence of new virulent phenotypes (Ivanova, 2012).

The resistance based on a single gene, is easy to overcome by a single mutation of the pathogen. Morgunov et al. (2011) presented data on cultivar Yuna, which has been widely grown in North Caucasus, and which became susceptible to the disease, within a 5-year period. This fact determined the specific resistance as not durable, but the problem may be overcome by constant introduction of new cultivars different by their genetic resistance to leaf rust. Gene pyramiding is a method for introduction of durable resistance in the plant. It has been established that 3-5 gene combinations can secure efficient resistance to the disease (Ladesma-Ramirez et al., 2018).

Highly significant for the breeding for resistance is the horizontal, or race non-specific resistance first described by Van der Plank (1968), it occurs in adult plants against all races of the pathogen, and is considered more durable, being controlled by several small genes with additive effect. There are different synonyms for this type of resistance: qualitative, race non-specific, partial, incomplete, *slow rusting*. The wheat cultivars with partial resistance to *P. triticina* allow the pathogen to sporulate, but to a much lower rate in comparison to the susceptible cultivars (Dyck & Kerber, 1985; Park & McIntosh, 1994; McIntosh et al.,1995). These genes determine the so-called durable resistance and their expression is related to slower, retarded development, longer latent period, smaller uredospores and lower number of spores (Basnet et al., 2013).

The adult resistance of wheat to this pathogen in Bulgaria was considered in detail by Donchev (1982). Later, Karzhin (1987) carried out wider and more thorough studies on the horizontal resistance of wheat. Stoyanov (1994) released a large number of cultivars with horizontal resistance according to the parameter "number of uredospores per unit leaf area at seedling stage and at heading stage. During 2000–2004 Todorova continued her studies on horizontal resistance (Karzhin, 2003). The experiments of Todorova & Andonova (2011) confirmed that this type of resistance was due to the cumulative effect of a longer latent period, lower infection frequency and lower sporulation rate.

The aim of this investigation was to determine the type of resistance of Bulgarian common and durum wheat cultivars developed at Dobrudzha Agricultural Institute to *P. triticina* under controlled conditions and in an infection field.

Material and Methods

During 2018 – 2020, 34 common winter wheat and 5 durum wheat cultivars developed at Dobrudzha Agricultural Institute – General Toshevo, Bulgaria, were tested for resistance to leaf rust (*P. triticina*). All cultivars possess good agronomy properties and are suitable for growing under the conditions of Bulgaria. The tested cultivars of common and durum wheat and their pedigrees are presented in Table 1.

The cultivars were sown in plots, each cultivar represented in 5 rows 1 m long, with 0.25 cm interspacing. The trial was carried out in two replications in an infection field. After every 10 rows of the cultivars were sown 2 rows of the multiplier *Michigan amber*. This cultivar was also sown along the plots for multiplication of the infection. The full set of virulent pathotypes identified during the respective year was propagated in the infection field.

The young plants infected with separate pathotypes were transplanted in the rows of the multiplier cultivar at tillering stage, and later at stage 32–36 (according to Zadoks et al., 1974) the inoculum was applied by injecting. The infection type was read according to the scale of Stackman et al. (1962). Infection types 0,0;, 1 and 2 were considered expression of the resistant type of reaction (R), while infection types 3, 4 and X were considered susceptible (S). The percent of attacked leaf area, or the final disease severity (FDS) for each cultivar was assessed with the help of the modified scale of Cobb (Peterson et al., 1948) at stage milk maturity. The level of resistance of the studied cultivars was estimated by comparing the average coefficient of infection (ACI) to the attacking rate of the standard susceptible cultivar by introducing constant values for each infection type (R – 0.2; MR – 0.4; M – 0.6; MS – 0.8; S – 1).

The resistance rate was estimated for each cultivar, as follows: Very resistant (VR) – ACI, or P_0 = 0 -5.99; Resistant (R) – P_0 = 6 – 25.99; Moderately resistant (MR) – P_0 =

Table 1. Pedigree of common and durum wheat cultivars

Cultivar	Pedigree
Common wheat	
Nicodim	Enola x Todora
Bozhana	Obriy x Milena
Aglika	2558-128 x Pliska
Stoyana	2477/2 x Slaveya
Rada	Enola x Preslav
Tina	Aglika x Tissa
Kristi	(Pryaspa x Miryana) x Enola
Karina	(Antilema 1-8 x Pliska) x Pliska
Korona	(Pryaspa x Olyivia) x (Manital x Flamura 80)
Karat	2811-2 x 371-2023
Kosara	Enola x Karina
Lazarka	Yuna x Flamura 85
Goritsa	F 302 – K_4-221-14 x Pobeda
Zhana	Pryaspa x Rufa
Kristalina	Yunak x Hersonskaya 86
Laska	F 2498-W1-2 x Obriy
Fani	(Aglika x Svilena) x 5626-5-2
Merilin	Lutescens 598 / 2852-21/ 5IWWSN-T-118-12
Neda	GP 2558-128 x 3746-2
Galateya	Pliska x 2367-8
Demetra	2185 – 51 x 1613/86-1
Sladuna	Laska x Flamura 85
Slaveya	Pliska / 2558-128 / Pliska
Kalina	(Prostor x Enola) x Prostor
Katarzhina	Enola x Kristal
Lider	11-8 x Trayana
Enola	518-4 x Hersonskaya 552
Kami	Yugtina x Flamura 85
Kiara	Klassik x Enola
Dragana	Miryana x Nadia
Iveta	Obriy x Mironovskaya 61
Pchelina	(Er. 2582-89-3 x 969 – 69) x Pegaso
Bolyarka	Yantar x F 2076W 12-11
Venka 1	754/90 x Dobrotitsa
Durum wheat	
Severina	Vitron x Aisberg Odeskiy
Mirela	F6 1162-5/ Parus x F6 1429-7-6/ Aisberg Odeskiy
Melina	(Leucurum 1079/93 x Sredets) x Hordeiforme 861/90
Mirabel	(Leucurum1107/92 x Harkovskaya 909) x Hordeiforme 861/90
Malena	Dnepryana x Belyi Parus

26 – 45.99; Moderately susceptible (MS) – P_0 = 46 – 65.99; Susceptible (S)- P_0 = 66 – 100.

Under conditions of a vegetation house, only the common winter wheat cultivars were tested to the separate pathotypes of *P. triticina* at seedling stage. Seven pathotypes of *P. triticina* were used: PHKTD, PKTTD, PKTTS, MKTTD, TKTTN, TKTTS, TKKTS. These pathotypes occurred with the highest frequency during last years (Ivanova, 2022 b; Ivanova et al., 2021). The inoculation of the plants was done at second leaf stage according to a standard methodology for working with rusts (Browder, 1971).

Of the statistical data processing was performed with software product Microsoft Excel, and the results were subjected to ANOVA to evaluate the strength of the effect of the sources of variation: genotype and environment.

Results and Discussion

Although, there has been a significant progress in the development of resistant varieties to this disease in Bulgaria, the frequent changes in the pathogen population still pose a considerable challenge to durable resistance. The pathogen monitoring provides evidence of constant changes occurring in the population and justify the necessity of coordinated efforts for the development and application of sustainable and efficient disease management strategies. Such monitoring is done annually at our laboratory, registering the population variability and the frequency of occurrence of each *P. triticina* pathotype identified in the respective year and the results are published in a number of papers (Todorova & Kiryakova, 2000; 2001; Kurzhin et al., 2003; Ivanova 2012; 2014; 2020; Ivanova, 2022a, b; Ivanova et al., 2021).

The race-specific resistance can be expressed at any stage of the host development; it is usually qualitative and relates to a programmed defensive response to cellular death known as hypersensitive immunity (Figlan et al., 2020). Most of the race-specific genes, however, cannot ensure the sufficient level of resistance defined by Jonson (1984) as the ability of the widespread gene for resistance to ensure a sufficiently high economic level of protection over a long period of time (Figlan et al., 2020).

In this research, aimed at finding the expression of race specificity, we investigated the response of common winter wheat cultivars to seven pathotypes of *P. triticina* under controlled conditions at seedling stage; the data are presented in Table 2.

The analysis of the results from the greenhouse investigation showed that most of the cultivars responded with a susceptible reaction to a large part of the pathotypes, and only some cultivars (Nikodim, Bozhana, Merilin, Iveta and

Table 2. Response of Bulgarian common winter wheat cultivars to separate *Puccinia triticina* pathotypes at seedling stage under greenhouse conditions

Cultivar	Pathotypes						
	PHKTD	PKTTD	PKTTS	MKTTD	TKTTN	TKTTS	TKKTS
Nicodim	S	R	R	S	R	S	R
Bozhana	R	S	S	R	R	S	S
Aglika	R	S	S	R	S	S	S
Stoyana	R	S	S	S	S	S	S
Rada	S	S	S	S	S	S	S
Tina	S	S	R	S	S	S	S
Kristi	S	S	S	S	S	S	S
Karina	S	S	S	S	S	S	S
Korona	S	S	S	S	S	S	S
Karat	S	S	S	S	S	S	S
Kosara	S	R	S	S	S	S	S
Lazarka	S	S	S	S	R	S	R
Goritsa	S	S	S	S	S	S	S
Zhana	S	S	S	S	S	S	S
Kristalina	S	S	S	S	S	S	S
Laska	S	S	S	R	S	S	S
Fani	S	S	S	S	S	S	S
Merilin	S	R	R	R	R	S	S
Neda	S	S	S	S	S	S	S
Galateya	R	R	R	S	S	R	S
Demetra	S	S	S	R	R	S	S
Sladuna	S	S	S	S	S	S	R
Slaveya	S	S	R	S	R	S	S
Kalina	S	S	S	S	S	S	S
Katardzhina	S	S	S	S	S	R	S
Lider	S	R	S	S	S	R	S
Enola	S	S	S	S	S	S	S
Kami	S	S	R	S	S	R	S
Kiara	R	S	R	S	S	S	S
Dragana	S	S	S	S	S	S	S
Iveta	R	R	S	S	S	S	R
Pchelina	S	S	S	S	S	S	S
Bolyarka	S	S	S	R	S	S	S
Venka 1	S	S	S	S	S	R	S
Michigan amber	S	S	S	S	S	S	S

Galateya) had a resistant reaction to three of four of the pathotypes. However, the response of these cultivars to the used pathotypes did not coincide with the response of any of the known genes for resistance to leaf rust, which have been identified up to now.

The resistance, provided by the APR genes is usually undiscoverable at seedling stage, but is usually efficient against a wide spectrum of physiological races, and is also durable (Figlan et al., 2020). In this relation, our investigation continued with plant pathology assessment of the common and durum wheat cultivars under the conditions of the infection field. The climatic conditions were favorable for the accumulation of leaf rust over the years of study, the attacking rate on the standard susceptible cultivar *Michigan amber* being within the range 90/4 – 100/4. The inoculum from the multiplier was transferred naturally to the tested cultivars. The parameters of field resistance – final disease severity (FDS), average coefficient of infection (ACI) and the resistance rate of the investigated cultivars are presented in Table 3.

Table 3. Field resistance of common and durum wheat cultivars under infection field during 2018–2020

Cultivar	2018			2019			2020		
Common wheat	Final rust severity	ACI	Rating	Final rust severity	ACI	Rating	Final rust severity	ACI	Rating
Nicodim	30/4	30.0	MR	40/4	44.4	MR	15/4	15.0	R
Bozhana	40/4	40.0	MR	25/4	27.8	MR	25/4	25.0	R
Aglika	30/4	30.0	MR	5/4	5.6	VR	25/4	25.0	R
Stoyana	0	0	VR	0	0	VR	5/4	5.0	VR
Rada	0	0	VR	5/4	5.6	VR	0	0	VR
Tina	25/4	25.0	MR	40/4	44.4	MR	25/4	25.0	R
Kristi	40/4	40.0	MR	40/4	44.4	MR	60/4	60.0	MS
Karina	40/4	40.0	MR	25/4	27.8	MR	40/4	44.4	MR
Korona	15/4	15.0	R	25/4	27.8	MR	0	0	VR
Karat	25/4	25.0	MR	60/4	66.7	MS	60/4	60.0	MS
Kosara	40/4	40.0	MR	60/4	66.7	MS	0	0	VR
Lazarka	40/4	40.0	MR	40/4	44.4	MR	40/4	40.0	MR
Goritsa	5/4	5.0	VR	25/4	27.8	MR	0	0	VR
Zhana	10/4	10.0	R	15/4	16.7	R	10/4	10.0	R
Kristalina	30/4	30.0	MR	40/4	44.4	MR	15/4	15.0	R
Laska	5/4	5.0	VR	60/4	66.7	MS	10/4	10.0	R
Fani	25/4	25.0	MR	10/4	11.1	R	25/4	25.0	R
Merilin	10/4	10.0	R	5/4	5.6	VR	5/4	5.0	VR
Neda	10/4	10.0	R	10/4	11.1	R	5/4	5.0	VR
Galateya	40/4	40.0	MR	10/4	11.1	R	30/4	30.0	MR
Demetra	10/4	10.0	R	5/2	2.2	VR	5/4	5.0	VR
Sladuna	25/4	25.0	MR	40/4	44.4	MR	25/4	25.0	R
Slaveya	10/4	10.0	R	30/4	33.3	MR	40/4	40.0	MR
Kalina	40/4	40.0	MR	40/4	44.4	MR	25/4	25.0	R
Katardzhina	25/4	25.0	MR	40/4	44.4	MR	15/4	15.0	R
Lider	10/4	10.0	R	10/4	11.1	R	0	0	VR
Enola	40/4	40.0	MR	40/4	44.4	MR	10/4	10.0	R
Kami	10/4	10.0	R	40/4	44.4	MR	15/4	15.0	R
Kiara	30/4	30.0	MR	5/4	5.6	VR	5/4	5.0	VR
Dragana	30/4	30.0	MR	40/4	44.4	MR	25/4	25.0	R
Iveta	10/4	10.0	R	5/4	5.6	VR	5/4	5.0	VR
Pchelina	25/4	25.0	MR	10/4	11.1	R	30/4	30.0	MR
Bolyarka	0	0	VR	5/4	5.6	VR	5/4	5.0	VR
Venka 1	10/4	10.0	R	0	0	VR	5/4	5.0	VR
Durum wheat									
Severina	5/4	5.0	VR	10/4	11.1	R	25/4	25.0	R
Mirela	40/4	40.0	MR	40/4	44.4	MR	25/4	25.0	R
Melina	25/4	25.0	MR	25/4	27.8	R	25/4	25.0	R
Mirabel	40/4	40.0	MR	40/4	44.4	MR	5/4	5.0	VR
Malena	10/4	10.0	R	25/4	27.8	MR	10/4	10.0	R
Michigan amber	100/4	100	S	90/4	100	S	100/4	100	S

The data from the field investigation revealed that cultivars Stoyana, Rada and Bolyarka were with very resistant reaction (VR) in all years of the investigation. Cultivar Zhana demonstrated resistant reaction (R) and in cultivars Karina and Lazarka moderately resistant reaction was observed (MR), the average coefficient of infection being within (ACI = 27.8 to 44.4). Common winter wheat cultivars Merilin, Neda, Iveta, Demetra, Lider, Venka-1 and the durum wheat cultivar Severina responded with very resistance to resistance of the type (VR-R). Very resistant to moderately resistant (VR-MR) were the common winter wheat cultivars Aglika, Korona, Goritsa, Kiara, and durum wheat Mirabel.

Very good resistance of the type (R-MR) was registered in the common winter wheat cultivars Nikodim, Bozhana, Tina, Kristalina, Fani, Galateya, Sladuna, Slaveya, Kalina, Katarzhina, Enola, Kami, Dragana, Pchelina and the durum wheat cultivars Melina, Mirela and Malena. These cultivars retard the development of the pathogen and probably this resistance could be maintained for a longer period of time since this type of resistance is not easily affected by the changes in the pathogen population. These cultivars allow the pathogen to affect the plant up to a moderate level without serious damages and this is the *slow rusting* type of resistance. It reduces the epidemic development of the disease although being a susceptible type of infection (Parlevliet & Van Ommeren, 1975).

A large part of the tested cultivars carry race non-specific (horizontal) resistance and this is explained by the fact that the parental components involved in the pedigree of most of the cultivars (Pliska, Pryaspa, Prostor, Yantur, Trayana, Enola, Aglika, Dobrotitsa, Flamura 80 and Flamura 85) are carriers of confirmed adult plant resistance (Kurzhin, 2003; Todorova & Andonova, 2004). In a previous investigation of ours, a part of the cultivars was studied with regard to the components of horizontal resistance – latent period, infection frequency and uredospore size under greenhouse conditions according to separate pathotypes of leaf rust, and with regard to the field parameters – area under the disease progress curve (AUDPC) and final disease severity (FDS) under infection field conditions (Ivanova, 2012). The final intensity of infection according to Gulati et al. (1985) is the better criterion for breeding of cultivars, which carry resistance of the *slow rusting* type.

Our previous investigations showed that a part of the cultivars, such as Merilin, Lazarka, Karina, Korona and Enola had a longer latent period than the one expressed by the control susceptible cultivar *Michigan amber* (Ivanova, 2012). In comparison to our studies from 2012, during this investigation the results showed that cultivar Merilin still maintained the tendency towards high resistance, while the field testing of cultivars Karina, Enola and Lazarka revealed that their resistance changed and varied over the years from resistant and moderately resistant to moderately susceptible.

In some of the cultivars, this change was probably due to the altered race composition of the pathogen and the occurrence of new more virulent pathotypes determined by the changes in the climatic factors, the mutations and migrations in the pathogen population. Cultivar Korona also maintained its level of resistance, demonstrating during this period very resistant to moderately resistant reaction (Table 3). The same type of resistance was exhibited also in cultivars Aglika, Goritsa, Kiara and the durum wheat cultivar Mirabel.

Some cultivars in the investigation responded in a wider range. Cultivars Kosara and Laska responded with very resistant to moderately susceptible reaction over the years (VR-MS), while cultivars Kristi and Karat were from moderately resistant to moderately susceptible (MR-MS).

Table 4 presents the two-way dispersion analysis used to evaluate the strength of the effect of the variation sources (genotype and environment) on the field parameters FDS and ACI of *P. triticina*. The results from the analysis showed that both the genotype and the environment influenced the field parameters (FDS) and (ACI) because for both of them $F > F$ crit at level of significance $P < 0.05$.

Table 4. Analysis of variance (ANOVA) for evaluation of the variation sources and effect on the field parameters FDS and ACI

Parameter	Sourses of Variation	SS	df	MS	F	P-value	F crit
	Genotype	34242.5	39	878.0128	6.171905	5.13E-12	1.553239
FDS	Year	1020.17	2	510.2083	3.586459	0.032354	3.113792
	Error	11096.25	78	142.2596			
	Total	46359.17	119				
	Genotype	37086.05	39	950.9244	5.981720	0.000000	1.553239
	Year	2001.85	2	1000.925	6.296245	0.002918	3.113792
ACI	Error	12399.8	78	158.9718			
	Total	51487.7	119				

$P < 0.05$

The results show that durum wheat cultivars are attacked to a lesser extent by leaf rust compared to common wheat cultivars.

The results from the field experiment also showed that the high level of resistance (VR) was established in 7.7% of the investigated cultivars. A stable resistance reaction (R) was found in 2.6% of the cultivars. Moderate resistance (MR) was registered in 5.1% of the studied cultivars. With highly resistant to resistant reaction responded 18% of the cultivars, and within the range of resistance to moderate resistance were 43.6% and 12.8% of the cultivars reacted from high resistance to moderately resistance. Some cultivars demonstrated variation within a wider range. The varieties responding with high resistance to moderate susceptibility (VR-MS) were 5.1%, and those reacting within the range of moderate resistance to moderate susceptibility (MR-MS) were also 5.1%.

Conclusion

Based on the results from the testing, the following conclusion can be drawn.

Under controlled conditions, at seedling stage, most of the cultivars demonstrated compatible susceptible response to the *P. triticina* pathotypes used in the study.

Under infection field conditions, adult plant resistance was present in the following cultivars: High resistance (VR) in cultivars Rada, Stoyana and Bolyarka; Stable resistance (R) in cultivar Zhana; Moderate resistance (MR) in cultivars Karina and Lazarka; Resistance of the type (VR-R) in the common winter wheat cultivars Merilin, Iveta, Neda, Demetra, Lider, Venka-1 and the durum wheat cultivar Severina; Resistance of the type (VR-MR) in cultivars Aglika, Korona, Goritsa, Kiara and the durum wheat cultivar Mirabel; Resistance of the type (R-MR) in cultivars Nikodim, Bozhana, Tina, Kristalina, Fani, Galateya, Sladuna, Slaveya, Kalina, Katarzhina, Enola, Kami, Dragana, Pchelina and the durum wheat cultivars Melina, Malena, Mirela.

The cultivars responding with incomplete resistance are to be preferred since this type of resistance remains relatively stable to the changes in the virulence spectrum of the pathogen and is often durable (Parlevliet & Zadoks, 1977).

Developing cultivars with efficient levels of durable resistance is a challenging and slow process. The cultivars, which demonstrated a certain type of resistance in this study (from high to moderate) may be included in the breeding programs for improvement of common and durum wheat according to the trait leaf rust resistance. The introduction of such cultivars can minimize the use of harmful chemical fungicides and can be used as an important component in the integrated management of pests; it is also a promising approach to sustainable agriculture.

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Received: **July, 18, 2022**; *Approved*: **August, 04, 2023;** *Published:* **December, 2023**