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Genetics and Breeding

Response of durum wheat lines to the cause agent of leaf rust *Puccinia triticina*

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Abstract. *The response of 1262 durum wheat lines to the cause agent of leaf rust Puccinia triticina was studied. The investigation was carried under infection field conditions during 2013-2015 at Dobrudzha Agricultural Institute (DAI) – General Toshevo, Bulgaria. Over the years of investigation, a maximum high infection background was developed by the cause agent of the disease, including all pathogen pathotypes identified during this period. The race variability in the population of the pathogen during the period was determined at the Plant Pathology Laboratory of DAI according to a validated methodology for working with rusts. Seventy-one phenotypically different virulent pathotypes were established. Fifteen new races of P. triticina were also identified, which have not been present during the previous 15 years in Bulgaria. Seventeen lines and one cultivar with a certain degree of resistance to the pathogen were selected. The selected lines can be involved in the breeding programs for developing initial material with resistance to leaf rust P. triticina.*

Keywords: adult plant resistance, durum wheat, infection, *Puccinia triticina*, virulent pathotypes

Introduction

Durum wheat is an important farming crop used primarily for the production of pasta and other extruded products. About 30 million ha are sown with this crop worldwide, and 30-35 million tons of grain are produced. The highest amounts of durum wheat are produced in the Middle East, followed by North America, Europe and North Africa. The main production in the EU is concentrated in the Mediterranean countries and was distributed in 2014 as follows: Italy - 46.4%, France - 21.6%, Spain - 16.5%, Greece - 13.5%. In Bulgaria, about 10% of the agricultural areas are sown with durum wheat (Dechev et al., 2010).

In recent years, the breeding of durum wheat in Bulgaria has been directed towards developing short-stemmed winter forms with increased cold resistance and resistance to lodging, and also with higher drought resistance related to the global climatic changes (Dechev et al., 2010; Alexandrov et al., 2019). Although the durum wheat cultivars are adapted to the changeable climatic conditions, their production is often limited by various fungal diseases (Nachit, 2000; Nsarellah et al., 2000; Singh et al., 2005).

Leaf rust caused by *P. triticina* is an important disease both on common and durum wheat *Triticum durum* Desf., it is also one of the biotic agents limiting production. Under favorable meteorological conditions, heavy epiphytotics occur, and the susceptible cultivars suffer significant losses of yield and quality (Cátedra, 2004; Herrera-Foessel et al., 2006; Dubin, 2009; Goyeau et al., 2012, 2014; Aoun, 2016; Singh et al., 2016). In Russia, the losses reach 40 percent and higher (Mikhailova et al., 2002; Kiseleva et al., 2016). Significant damages are reported from the Volga River region, Central and North Caucasus and Ural. Long-term studies in West Siberia show that the rust attacks on durum wheat have increased drastically during the last 20 years (Scolotneva, 2018).

Up to 75% of losses are reported from the territory of Ethiopia in instances of severe infection (Shimelis and Pretorius, 2005; Badebo et al., 2008). Since the beginning of 2000, increased susceptibility of durum wheat to leaf rust has been observed worldwide, including the Mediterranean region. In 2001, in the North-West part of Mexico, and later in California and Kansas (USA), virulent races were identified, which overcame the resistance of the widely adapted durum wheat cultivars developed at the International Center for improvement of maize and wheat (CIMMYT), which were maintaining resistance for more than 25 years (Aoun, 2016).

By the end of the 20th century, the knowledge on the genetic basis of resistance to leaf rust in durum wheat was comparatively limited in comparison to common wheat (Kolmer, 1996). Later,

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researchers working in this field (Singh et al., 2004; Herrera-Foessel et al., 2005) emphasized the necessity of resistant germ plasma in durum wheat and better understanding of its genetic basis.

The developing and growing of resistant genotypes is an on-going process in the breeding of resistance to diseases, to which it is necessary to add new efficient sources to widen the genetic basis and to achieve diversification that would ensure high resistance to biotic types of stress, including leaf rust (Arain et al., 2017).

This disease occurs annually in all regions of Bulgaria, the attacks of leaf rust getting more severe in the recent years. Although fungicides can control the disease, researches are being carried out to find ways for developing durable resistance in the genotypes, especially now, when the national and European goals are set towards limited use of pesticides and the attention is directed towards the application of methods for production of clean foods in accordance with the European priorities of food safety (Lalev and Kiryakova, 2004; Martinez et al., 2007; Plamenov et al., 2009; Soleiman et al., 2014). With the occurrence of new *P. triticina* races, the resistance of the present durum wheat cultivars is at risk and therefore there is a clear necessity of checking and knowing the level of resistance of the initial material and the new developed varieties.

The aim of this investigation was to determine the level of resistance in the new durum wheat lines developed at Dobrudzha Agricultural Institute - General Toshevo in dependence on the changed structure of the *P. triticina* pathogen population in Bulgaria during the recent years.

Material and methods

The investigation was carried out during 2013-2015 in the experimental field of Dobrudzha Agricultural Institute (DAI) – General Toshevo, located in the eastern part of the Dobrudzha Plateau, at latitude 43°N and longitude 28°E, 236 m above sea level. The new developed durum wheat lines were subjected to evaluation for leaf rust resistance for three seasons, under infection field conditions. Out of the 1262 durum wheat lines tested for resistance to the cause agent of leaf rust (*P. triticina*), 17 lines and one variety were selected. They were assessed by degree of resistance, from high to moderate.

The investigation was carried out under conditions of maximum infection developed in a field where the full set of virulent pathotypes identified for the respective year were released. The lines were sown manually, in 1.5 m rows with 0.25 m interspacing. Cultivar *Michigan amber* was used for propagation and distribution of the infection; it was sown transversely at every 10 breeding lines, and along the plots as well. The artificial inoculation with the pathogen was done according to the methodology used at the Plant Pathology Laboratory of DAI (Ivanova, 2012).

The type of infection and the attacking rate were read according to the scale of Cobb, modified by Peterson (1948), at milk maturity stage. The average coefficient of infection (ACI), or the so-called corrected relative attacking rate, was calculated by introducing a coefficient for the respective infection type (R-0.2; MR-0.4; M-0.6; MS-0.8; S-1). International Center for improvement of maize and wheat (CIMMYT) has found the ACI to be a useful method for ranking or rating varieties (Stubbs et al., 1986). Depending on the values of ACI, the studied lines were divided into several groups: immune (ACI=0); very resistant - VR (ACI=0-5.99); resistant - R (ACI=6-25.99); moderately resistant - MR (ACI=26-45.99); moderately susceptible - MS (ACI=46-65.99) and susceptible - S (ACI=66-100). The lines with susceptible reaction were of no interest to us.

Results and discussion

One of the challenges in the breeding for resistance to diseases, to *P. triticina* in particular, was that the pathogen quickly acquired new aggressive virulence, which overcame the race-specific genes (Pretorius et al., 2015; Boshoff et al., 2018). This imposed the necessity to annually study and register the changes in the pathogen population. Our researches showed that during 2013-2015 the *P. triticina* population was rather variable and included a large number of virulent pathotypes identified at the Laboratory of Plant Pathology (Table 1). In 2013, 27 pathotypes were identified, 15 of them being new ones, not identified during the past 15 years. The predominant pathotypes in 2013 were 12762 - 16.4%; 52762 - 15%; 42762 and 52562 - 9.6%. In 2014, 37 phenotypically different pathotypes were identified, pathotype 53763 being the leading one in the race composition during that year with 12.1% of distribution, followed by pathotypes 12763 - 9.1% and 33763 - 7.6%. In 2015, 25 phenotypically different pathotypes were identified, and 13763 - 17.4%, 12763 - 8.7% and 73763 - 7.3% were with the highest percentage of distribution. The full variability of the 71 virulent, phenotypically different pathotypes established over the years of investigation were transferred to the infection field.

In addition to the favorable meteorological conditions, the changes in the virulence of the pathogen population in combination with the high susceptibility of the cultivars, were the main factors, which conditioned the distribution of the disease (Arain et al., 2017).

There is an increasing amount of data in the literature sources indicating the occurrence of new races, which have overcome the resistance in durum wheat. Huerta Espno et al. (2011) reported the loss of resistance in the combination of genes *Lr* 27+31 in 2008 in the previously resistant cultivars “Jupare C2001” and “Banamichi C2004” with the occurrence of the new race BBG/BP in Sonora State, Mexico. In research on the leaf rust attack on durum wheat in Spain during 2009-2011, Soleiman (2013) presented data that all races of *P. triticina* were avirulent on durum wheat cultivars, which carried gene *Lr* 14a, and avirulent on cultivar Altar carrying gene *Lr* 72, while Goyeau et al. (2012) pointed out that these genes were no longer effective in France. Later, there were reports on virulence occurring on these genes in Spain and Tunisia (Gharbi et al., 2013; Loladze et al., 2014). Boshoff et al. (2018) reported higher frequency of the new identified races, which were overcoming the resistance of genes *Lr* 3, *Lr* 15, *Lr* 20 and *Lr* 26, which were detected in the most widely distributed South African durum wheat cultivars. In Ethiopia, Tesfaye et al. (2020) reported the occurrence of three new pathotypes, BBBB, BBBN and BBBQ. The authors commented that race BBBN was discovered for the first time in Ethiopia, but was less virulent on the durum wheat cultivars than races BBBQ and BBBB, while phenotype BBBB had relatively high virulence on the Ethiopian durum wheat cultivars, infecting about 42.5 % of the tested varieties.

Table 1. Race variability of *P. triticina* determined during 2013-2015

Pathotype	2013, %	2014, %	2015, %	Pathotype	2013, %	2014, %	2015, %
02560	1.4	-	-	42762	9.6	-	-
02562	1.4	-	-	42763	1.4	-	-
02762	2.7	1.5	-	52560	5.5	-	-
10762	1.4	-	-	52562	9.6	-	-
12560	2.7	-	-	52563	1.4	-	-
12562	1.4	-	-	52760	5.5	-	-
12663	-	1.5	-	52762	15.0	1.5	-
12702	1.4	-	1.4	52773	-	1.5	-
12722	1.4	3.0	5.8	53762	-	3.1	7.3
12723	-	1.5	4.3	53763	-	12.1	7.3
12760	1.4	-	-	53773	-	1.5	-
12766	-	-	3.0	53722	-	-	1.4
12762	16.4	6.1	3.0	53723	-	1.5	1.4
12763	2.7	9.1	8.7	62562	1.4	-	-
12733	-	1.5	-	63563	-	1.5	-
43773	-	-	1.4	63763	-	-	3.0
12773	-	1.5	-	63776	-	1.5	-
13723	-	-	5.8	50763	-	-	1.4
13743	-	-	1.4	53767	-	-	1.4
43763	-	-	1.4	50742	-	-	1.4
13762	-	1.5	5.8	71772	-	1.5	-
13763	-	1.5	17.4	72167	1.4	-	-
13773	-	1.5	-	72560	1.4	-	-
16362	-	1.5	-	72564	1.4	-	-
22773	-	1.5	-	72760	-	1.5	-
32722	1.4	-	-	72763	-	1.5	-
32762	-	1.5	-	72765	1.4	-	-
32763	-	3.1	-	72722	-	1.5	-
32773	-	1.5	-	72767	1.4	-	-
33573	-	1.5	-	73562	-	1.5	-
33762	-	1.5	1.4	73763	-	6.1	7.3
33763	-	7.6	-	73772	-	3.1	-
42562	5.5	-	-	73773	-	1.5	-
42760	2.7	-	-	73767	-	1.5	3.0
73746	-	1.5	-	43762	-	-	3.0
73322	-	-	1.4	-	-	-	-

In Bulgaria, higher attacking rates of leaf rust on new durum wheat lines and varieties are being observed with increasing frequency (data not published). Anpilogova et al. (2009) expressed the opinion that the success of a breeding program for developing cultivars with durable rust resistance is possible on the basis of a wide genetic variability of the initial material, simultaneously taking account of the intraspecific differentiation of the pathogen and the tendencies of the changes occurring.

In this study on the resistance to the cause agent of rust *P. triticina*, out of the tested 1262 breeding lines, 17 lines and 1 variety were selected. Table 2 presents the pedigree of the lines, and Table 3 – the resistance of these lines under conditions of an infection field.

The investigated materials - cultivars and promising breeding lines, were typical durum wheats, i.e. belonging to the species *Triticum durum* Desf., obtained through the method of intervarietal hybridization. The pedigree of the breeding lines and varieties originated at Dobrudzha Agricultural Institute, involves varieties and promising lines from different geographic origins, which have differences in their genetic potential determining different traits and properties. These breeding materials are of winter type of development and possess high and stable winter and cold resistance, low stem resistance to lodging, increased number of productive tillers and excellent grain quality. The durum wheat lines and varieties developed at DAI, including line 2200-3/06, which was later released as cultivar *Malena*, include genetic material from durum wheats of Ukrainian and Russian breeding such as cultivars *Aisberg Odeskiy, Parus, Alyi parus, Dnepryana, Belyi parus, Harkovskaya 909* and *Leucurum 21,* or promising breeding lines: *Leucurum 1079/93, Leucurum 1704/94, Leucurum 1107/92, Leucurum 1441/93, Hordeiforme* 861/90, *Hordeiforme* 1145/93, which carry positive biological properties and traits, including resistance to leaf rust. Some of the lines involved in the crosses of the new breeding lines such as 1642-11/01, 533-9/08 and 594-8/98 include genetic material from durum wheats with origin from North Macedonia and Russia. In these breeding lines, regardless of the presence of alien genetic material from the species *Triticum turgidum* L. or *Triticum aestivum* L., selection was carried out in the hybrid populations towards obtaining of breeding products of the type *Triticum durum* Desf. The parental forms participating in the new lines are carriers primarily of field resistance to leaf rust, which explains the manifested type of resistance in these lines.

Table 2. Pedigree of the selected with manifested resistance lines

Cultivar/line	Pedigree
Severina	Vitron x Aisberg Odeskiy
551-15 D/07	Vitron x Aisberg Odeskiy
37/18-4*-15	Yavaros 79 x Aisberg Odeskiy
56/18-4 n	(Exodur x Aisberg Odeskiy) x Aisberg Odeskiy
62/16-12 a/03	(Yavaros 79 x Alyi Parus) x Alyi Parus
2165-70/05	(Leucurum 1079/93 x) x Sredets) x Hordeiforme 861/90
3170/13-3**-1**-5/09	1642-11/01 x Hordeiforme 922/93
2174-63/06 B	F_1 2012 (Leucurum 1704/90 x Harkovskaya 909) x Hordeiforme 861/90
2175-21/05	F_1 2012 (Leucurum 1704/90 x Harkovskaya 909) x Leucurum 21
2200-3/06	Dnepryana x Belyi Parus
2635-1/06	F_1 2380 (533-9/08 x Hordeiforme 1145/93) x Saturn 1
2636-14/07	F_1 2380 (533-9/08 x Hordeiforme 1145/93) x 981/90
2643-16/08	(594-8/98 x Leucurum 1441/93) x Parus
2729-10/08	Beloslava x 551-5
2787-13/07	Aisberg Odeskiy x Saturn 1
2169-12/07	(Leucurum 1079/93 x Aisberg Odeskiy) x Leucurum 21
26/16-3 S_{11} Y/04 F	Agridur x Alyi Parus
2165-101/05	(Leucurum 1079/93 x) x Sredets) x Hordeiforme 861/90

Table 3 presents the field resistance of the breeding lines to *P. triticina* and the main parameters characterizing the resistance of the lines – final rust severity, corrected value and rating. The attack on the universally susceptible cultivar *M. amber* was 70-100% during the period of testing.

In their researches, Singh et al. (1993) presented data indicating that cultivar Yavaros 79 is susceptible at seedling stage to individual leaf rust races but has very good adult slow rust resistance as a result of the presence of two effective genes with additive effect. This was confirmed in our investigations, too, since the lines involving this cultivar (37/18-4*-15 and 62/16-12a/03) demonstrated very good field resistance. The first line showed durable resistant reaction during the three years of the investigation, and the second line responded as very resistant in two of the years and as resistant during the first year. Researches for finding out resistant gene plasma are being constantly carried out in different parts of the world. Herrera-Foessel et al. (2008) reported the discovery of a high level of partial resistance in some durum wheat lines. Loladze et al. (2014) found resistance in durum wheat varieties from different origins – Gaza, Amria, Geromtel_3, Geruftel_1, Tunsyr_2 and Biblos. In Spain, Martinez and Solis (2019) studied 917 accessions from the Spanish Center of Genetic Resources (CRF- INRA). Most of the investigated accessions showed susceptible reaction and only 4.8% of the durum wheat turned out to be resistant.

The results from our study showed stable very resistant reaction (VR) in three lines - 2635-1/06, 2636-14/07 and 2169-12/07. Lines 551-15D/07, 56/18-4n, 62/16-12a/03, 3170/13-3**-1**-5/09, 2175-21/05, 2643-16/08 and 2729-10/08 responded with resistance (R) to high resistance (VR). Durable resistance (R) in the three years of the study was demonstrated by cultivar Severina and lines 37/18-4*-15, 2200-3/06, 2787-13/07 and 2165-101/05. The response of lines 2174-63/06 B and 27/16-39-y/046 varied from high to moderate resistance (VR-MR), and the response of one line (2165-70/05) was within the range R-MR.

Table 3. Adult plant resistance of *durum* wheat lines to *P. triticina*

Cultivar/Line	2013			2014			2015		
	Final rust severity	ACI*	Rating	Final rust severity	ACI	Rating	Final rust severity	ACI	Rating
Severina	5/4	6.25	R	5/4	6.3	R	5/4	7.1	R
551-15D/07	5/4	6.25	R	0	0	VR	0	0	VR
37/18-4*-15	5/4	6.25	R	10/4	12.5	R	5/4	7.1	R
56/18-4n	5/4	6.25	R	0	0	VR	10/4	14.3	R
62/16-12a/03	5/4	6.25	R	0	0	VR	0	0	VR
2165-70/05	5/4	6.25	R	25/4	31.3	MR	5/4	7.1	R
3170/13-3**-1**-5/09	5/4	6.25	R	0	0	VR	5/4	7.1	R
2174-63/06 B	0	0	VR	10/4	12.5	R	25/4	37.6	MR
2175-21/05	0	0	VR	0	0	VR	5/4	7.1	R
2200-3/06	5/4	6.25	R	5/4	6.3	R	10/4	14.3	R
2635-1/06	0	0	VR	0	0	VR	0	0	VR
2636-14/07	0	0	VR	0	0	VR	0	0	VR
2643-16/08	5/4	6.25	R	0	0	VR	0	0	VR
2729-10/08	5/4	6.25	R	0	0	VR	0	0	VR
2787-13/07	10/4	12.5	R	10/4	12.5	R	10/4	14.3	R
2169-12/07	0	0	VR	0	0	VR	0	0	VR
27/16-39-y/046	25/4	31.3	MR	25/4	31.3	MR	0	0	VR
2165-101/05	5/4	6.25	R	10/4	12.5	R	5/4	7.1	R
M. amber	70/4	100	VS	100/4	100	VS	90/4	100	VS

*ACI- average coefficient of infection

Conclusion

As a result of the investigation carried out, it was found out that the population of *P. triticina* during 2013-2015 was rather variable and was represented by 71 phenotypically different pathotypes. Pathotypes 13763 (17.4%), 12762 (16.4%) and 53763 (12.1%) were predominant in the pathogen's population during this period. Fifteen new pathotypes were identified, which were not detected in the previous 15 years. The obtaining of new initial material – carrier of valuable economic traits, including disease resistance, is a major task and an on-going process in every breeding program. Out of the 1262 durum wheat lines, 17 lines and one cultivar were selected, which demonstrated stable resistance to *P. triticina* during the investigated period. The high to moderate resistance exhibited by the studied lines was of race non-specific nature and can provide sufficient protection of the host to *P. triticina*. These data can direct the attention of breeders to the development of suitable conventional and advanced technologies to produce improved varieties with a wide range of durable resistance to the existing and new emerging races of *P. triticina*.

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