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Original article

New Common Winter Wheat Lines with Resistance to Leaf Rust (*P. triticina*) [1]

Vanya Ivanova [a, *], Galina Mihova [a] & Bilyana Ivanona [a]

[a] Dobrudzha Agricultural Institute – General Toshevo 9520, Bulgaria

Abstract

Wheat is the most important cereal crop in global agricultural economy and is cultivated in diverse agroclimatic regions of the world. Breeding for disease resistance is the most economically and environmentally safe method to reduce crop losses. The long term success of breeding for disease resistance depends on the nature of the pathogen and the virulence spectra in the pathogen population, the availability, diversity and type of genetic resistance in the host and the methodology for screening and selection for resistance.

During 2013-2015, under conditions of an infection field at Dobrudzha Agricultural Institute-General Toshevo, Bulgaria, 680 common winter wheat lines were tested to a population of races of leaf rust *P.triticina*. Forty-two of the lines, which exhibited resistance under field conditions, were tested to 9 pathotypes of the pathogen at seedling stage under controlled conditions. Some of the tested lines carried adult plant resistance (APR), while others had combination of race specific and race nonspecific resistance.

The combination of various mechanisms of resistance is of great importance for the durability of resistance. In this relation, the investigated 42 lines can be considered efficient sources of resistance which can be used in the breeding programs.

Keywords: Wheat, *P. triticina*, Sources of resistance, Race specific resistance, Race nonspecific resistance

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* **Corresponding author:**

Vanya Ivanova, Dobrudzha Agricultural Institute – General Toshevo 9520, Bulgaria
Email: vkiryakova@yahoo.com

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INTRODUCTION

Leaf rust is the most common disease on wheat, which causes high yield losses (Huerta Espino et al., 2011). The importance of this disease depends on the spread of highly aggressive and virulent races of the pathogen and on their compatibility with the genetic constitution of the host. The early infection with leaf rust can lead to yield losses exceeding 50 %. Developing and growing resistant cultivars is one of the most important methods to reduce the yield losses. The resistance is determined by the interaction of the host and the pathogen, the conditions of the environment, the stage of host development and the interaction between the genes for resistance in the wheat genome (Kolmer,1996). Over eighty genes for resistance to brown rust are currently known, which provide specific resistance to individual races of the pathogen based on the "gene for gene" principle (McIntosh et al., 2007). Nevertheless, the cultivars, which carry race-specific resistance, often quickly lose their efficiency within several years. The cultivar carrier of a single gene for resistance can rapidly lose its resistance because it is overcome by a single mutation of the pathogen. The erosion of the genes for specific resistance imposes the necessity to search for alternative approaches for management of the resistance. The qualitative race nonspecific resistance reduces the attacking rate, i.e. there is presence of the pathogen but not lack of the disease; the plant is attacked to a moderate level and in this case there is no breeding pressure on the pathogen and it is not stimulated to form new virulent races. This resistance is based on the action of several genes with partial effect. This type of resistance is efficient against all races of the pathogen and occurs at adult plant stage.

Some empirical results show that the qualitative resistance can be more durable than the full resistance (Mundt et al., 2002; Parleviet, 2002).

Kumar et al. (2015) point out that developing cultivars with durable resistance based on small genes is a challenge and is often a slower process due to the following reasons:

- Genotypes with sufficient number of small genes are not available;
- The genotype carrier of such genes may be poorly adapted;
- Confounding effects from the segregation both of the main and the secondary genes in the population are also possible;

The schemes of crossing and selection and the sizes of the populations are more suitable for selection of main genes rather than of secondary ones, but the transfer of these small genes to adapted landraces will be very useful.

This investigation was aimed at studying the response of new common winter wheat lines at young stage to single races of the pathogen, and at adult stage to the population of *P.triticina*. The lines, which demonstrated stable resistance, can be used as parental components in the development of new wheat cultivars resistant to leaf rust.

Material and Methods

During 2013 – 2015, 680 common winter wheat lines from a competitive varietal trial were tested in the infection field of DAI – General Toshevo. Out of them, 42 lines were selected for resistance to the cause agent of brown rust P. triticina; these lines responded with a certain degree of resistance (MR-VR) in the infection field and demonstrated resistant reaction to some of the used pathotypes at young stage under greenhouse condition. The investigation was carried out under conditions of maximum infection created in the infection field, where the set of pathotypes, identified for the respective year, was previously multiplied. The lines were sown manually in 1.5 wide rows, with 0.25 cm interspacing. The cultivar *M. amber* was used as a multiplier and carrier of leaf rust. 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 stage milk maturity. The average coefficient of infection (ACI), or the so-called corrected relative attacking rate, was calculated by introducing a coefficient for each of the infection types (R-0,2; MR-0,4; M-0,6; MS-0,8; S-1). Depending of the values of ACI, the studied lines were divided into several groups: immune (ACI=0); VR (ACI= 0-5,99); R (ACI = 6-25,99); MR (ACI= 26-45,99); MS (ACI= 46-65,99); S (ACI = 66 - 100).

The lines, which demonstrated susceptible reaction, were of no interest to this study. The lines with high to moderate resistance (VR-MR) were selected. The forty-two lines, which showed certain degree of resistance in the infection field, were subjected to multi-pathotype testing with nine pathotypes of different virulence under controlled climatic conditions, in compliance with the standard procedures (Browder, 1971). The pathotypes, used in the testing, were identified on the basis of 15 monogenic lines: *Lr1*, *Lr 2a*, *Lr 2b*, *Lr 2c*, *Lr 3, Lr 9, Lr 11, Lr 15, Lr 17, Lr 19, Lr 21, Lr 23, Lr 24, Lr 26* and *Lr 28*, which are presented in Table 1 and which were coded by the method of Limpert and Muller (1994).

Table 1. Isogenic lines used for pathotype differentiation

Lr genes	Pedigree	Origin	ID number
Lr 1	Tc*6/ Centenario	Wheat	RL 6003
Lr 2a	Tc*6/ Webster	Wheat	RL 6016
Lr 2b	Tc*6/ Carina	Wheat	RL 6019
Lr 2c	Tc*6/ Loros	Wheat	RL 6047
Lr 3	Tc*6/ Democrt	Wheat	RL 6002
Lr 9	Transfer/Tc*6	*Aegilops umbellulata*	RL 6010
Lr 11	Tc*2/ Hussar	Wheat	RL 6053
Lr 15	Tc*6/ Kenya W 1483	Wheat	RL 6052
Lr 17	Klein Lucero/ Tc* 6	Wheat	RL 6008
Lr 19	Tc*7/Translocation 4	*Agropyron elongatum*	RL 6040
Lr 21	Tc*6/RL5406 x RL529	Wheat	RL 6043
Lr 23	Lee 310/ Tc*6	Triticum turgidum var.durum	RL 6012
Lr 24	Tc*6/ Agent	*Agropyron elongatum*	RL 6064
Lr 26	Tc*6/ St-1-25	*Secale cereale*	RL 6078
Lr 28	Tc*6/ C-77-1	*Aegilops speltoides*	RL 6079

To improve the formation of spores, the plants were treated with solution of Maleic hydrazide 97% (1g per 3 l water). On the 9^{th}- 12^{th} day after inoculation, the type of infection was read according to the scale of Stakmen et al. (1962) presented in Table 2.

Table 2. Scale for reading *P.triticina* infection type under greenhouse condition at young stage

Infection type	Reaction	Symptoms
0	Immune	No uredospores or other macroscopic symptoms of infection.
0;	Close to immune	No uredospores, but some supersensitive necrotic or chlorotic spots.
1	Very resistant	Small uredia surrounded by necrosis.
2	Moderately resistant	Small to medium-sized uredia surrounded by chlorosis or necrosis.
3	Moderately susceptible	Large uredospores without chlorosis or necrosis
4	Very susceptible	Large uredospores without chlorosis or necrosis.
X	Heterogeneous	Random distribution of uredospores of variable size on single leaves.

Infectious types 0, 1 and 2 were considered expressions of a resistant type of reaction (R), while infectious types 3, 4 and X were considered susceptible (S).

Results and Discussion

The genetic resistance is expressed through two main classes of genes based on their phenotypic effects – genes for specific resistance, which are manifested as resistant genes to individual pathotypes of the pathogen at young stage, and genes for resistance at adult stage (APR).The analysis of our study was done in this direction: to what extent the investigated 42 lines were carriers of one of the two types of genetic resistance, or did they possess race non-specific type of resistance. Among the studied lines, line 11060/12-305 at young stage demonstrated resistant reaction only to pathotype 72167 (Table 3). The field reaction showed that during the years of study it responded as resistant to moderately resistant line (Table 4). Depending on the way it reacted, we can affiliate it to the lines with expresses field resistance of the slow rusting type.

Table 3. Reaction of common winter wheat lines to nine pathotypes of *P. triticina* in seedling

Cultivar/Lines	Pathotypes of *P.triticina*								
	12762	42762	52762	72167	53763	12763	72760	16362	53723
11060/12-305	S	S	S	R	S	S	S	R	S
11161/12-628	S	S	S	R	S	S	S	S	S
11161/12-630	S	R	S	S	S	S	S	S	S
11161/12-637	S	S	S	S	S	S	S	R	S
11169/12-665	S	S	S	S	S	S	S	S	S
10234/10-288/12-126	S	R	S	S	S	S	S	S	S
10252/10-325/12-150	S	S	S	S	S	S	S	S	S
21HRWSN/12-910	R	R	S	R	S	R	S	S	S
9600-85-17/12-5	S	S	S	R	S	S	S	S	S
8118-61-25	S	R	S	S	S	S	S	S	S
10320-113/11-127	S	S	S	R	S	S	S	S	S
10337-110/11-143	S	S	S	R	S	S	S	S	S
10552/11-187	S	R	R	S	S	S	S	S	S
10792/11-267	R	R	S	R	S	S	S	R	S
11144/11-379	S	R	S	S	S	S	S	S	S
10241/11-449	S	R	S	S	S	S	S	S	S
11099	S	S	S	S	S	S	S	S	S
11260	S	S	S	S	S	S	S	S	S
3059-1-4	S	R	S	S	S	S	S	S	S
3059-1a-2	S	R	S	R	R	S	R	S	S
4340-1-1	S	S	S	R	S	S	S	S	S
4296-1-2	S	S	S	S	S	S	S	R	S
5227-2	S	S	S	S	S	S	S	S	S
3584-9-10	S	S	S	R	S	S	S	S	S
3745-1a-2	S	R	S	S	S	S	S	S	S
721c/63-3-2	S	R	S	S	S	S	S	R	S
3059-1-1-2б	S	R	S	R	S	S	S	S	S
3326-1-1б	R	R	R	R	S	S	S	S	S
3548-4-1б	S	S	S	R	S	S	S	S	S
4340-4-1	S	S	S	S	S	S	S	S	S
01/165-91	S	S	S	S	S	S	S	R	S
S11/631	S	S	S	S	S	S	S	S	S
2026-1-811-97	S	S	S	S	S	S	S	R	S
01/29-81-101	S	R	S	S	S	S	S	R	S
01/29-94-1021-21	S	S	S	S	S	S	S	S	S
05N12-21	S	S	S	S	S	S	S	S	S
05N26-21	S	S	S	S	S	S	S	S	S
05N57-22	S	S	S	S	S	S	S	S	S
05N83-22	S	S	S	S	S	S	S	S	S
06N139-22	R	R	S	S	R	S	S	S	S
06N153-23	S	R	S	S	S	S	S	S	R

06N50-21	R	S	S	S	S	S	S	S	S
M. amber	S	S	S	S	S	S	S	S	S

Line 11161/12-630 demonstrated stable resistance under field conditions during the three years of study, while line 11161/12-630 showed resistance of the type VR-R. All three lines were carriers of resistance of race non-specific type (Table 4). A part of the lines, 11169/12-665, 10234/10-288/12-126, 10252/10-325/12-150, 9600-85-17/12-5, 11260, 4296-1-2, 721c/63-3-2, 3059-1-1-2б, 3548-4-1б, 01/29-94-1021-21, 05N12-21, 05N57-22, 05N83-22 and 06N153-23, had similar response at young stage and showed susceptible reaction to all pathotypes used under greenhouse conditions, or demonstrated resistant reaction only to one or two pathotypes, and in the field responded with resistance of the type VR – MR. These Lines 11161/12-628, 11161/12-630 and 11161/12-637 were obtained from the same cross but differed a little between themselves, with only slight variations. Line 11161/12-628 at young stage responded with a resistant reaction to pathotype 72167, line 11161/12-630 demonstrated resistance to pathotype 42762, and line 11161/12-637 was resistant to pathotype 16362 (Table 3). Analyzing the field reaction of line 11161/12-628 – it demonstrated type of resistance VR-MR, i.e. it is a carrier of the *slow rusting* type of resistance.

lines allowed the pathogen to attack the host to a moderate level, which implied its stunted development and inability to exercise breeding pressure on it. All these lines carried field resistance of the *slow rusting* type (Table 4).

Another group of lines at young stage responded with a susceptible reaction to all pathotypes, or with a resistant reaction to one or two pathotypes, and the field reaction was of the type VR – R. Such reaction was demonstrated by lines 8118-61-25, 10320-113/11-127, 10337-110/11-143, 10552/11-187, 11144/11-379, 10241/11-449, 11099, 3059-1-4, 3059-1a-2, 4340-1-1, 5227-2, 3584-9-10, 3745-1a-2, 3548-4-1б, 01/165-91, 01/29-81-101, 05N26-21, 06N50-21. All these lines carried race non-specific resistance, i.e. at adult stage they exhibited resistance to all races of the pathogen.

Another five lines, 21HRWSN/12-910, 10792/11-267, 3745-1a-2, 3326-1-1б and 06N139-22, at young stage responded with resistant reaction to 3 or 4 pathotypes, and the field reaction was of the type VR-R, R-MR or VR-MR. It can be assumed, that these lines carry partial race specific resistance, but to prove this type of resistance more detailed breeding and genetic studies are necessary, and they are not the subject of this investigation.

Table 4. Adult plant response

Cultivar/Lines	2013			2014			2015		
	Final rust severity	ACI	Rating	Final rust severity	ACI	Rating	Final rust severity	ACI	Rating
11060/12-305	5/4	6.3	R	10/4	12.5	R	25/4	37.6	MR
11161/12-628	0	0	VR	30/4	37.5	MR	10/4	14.3	R
11161/12-630	5/4	6.3	R	5/4	6.3	R	5/4	7.1	R
11161/12-637	0	0	VR	0	0	VR	5/4	7.1	R
11169/12-665	15/4	18.8	R	0	0	VR	25.4	37.6	MR
10234/10-288/12-126	5/4	6.3	R	25/4	31.2	MR	25.4	37.6	MR
10252/10-325/12-150	10/4	12.5	R	10/4	12.5	R	25/4	37.6	MR
21HRWSN/12-910	5/4	6.3	R	0	0	VR	0	0	VR
9600-85-17/12-5	5/4	6.3	R	0	0	VR	25/4	37.6	MR
8118-61-25	10/4	12.5	R	10/4	12.5	R	0	0	VR
10320-113/11-127	5/4	6.3	R	0	0	VR	10/4	14.3	R
10337-110/11-143	0	0	VR	0	0	VR	5/4	7.1	R
10552/11-187	10/4	12.5	R	5/4	6.3	R	5/4	7.1	R
10792/11-267	10/4	12.5	R	5/4	6.3	R	0	0	VR
11144/11-379	5/4	6.3	R	10/4	12.5	R	0	0	VR
10241/11-449	15/4	18.8	R	0	0	VR	10/4	14.3	R
11099	5/4	6.3	R	5/4	6.3	R	5/4	7.1	R
11260	25/4	31.2	MR	5/4	6.3	R	5/4	7.1	R
3059-1-4	0	0	VR	0	0	VR	5/4	7.1	R
3059-1a-2	0	0	VR	0	0	VR	10/4	14.3	R
4340-1-1	0	0	VR	5/2	2.5	VR	10/4	14.3	R
4296-1-2	5/4	6.3	R	5/2	2.5	VR	25/4	37.6	MR
5227-2	5/4	6.3	R	0	0	VR	0	0	VR
3584-9-10	5/4	6.3	R	15/4	18.8	R	10/4	14.3	R
3745-1a-2	0	0	VR	0	0	VR	10/4	14.3	R
721c/63-3-2	10/4	12.5	R	25/4	31.2	MR	10/4	14.3	R
3059-1-1-2б	0	0	VR	0	0	VR	25/4	37.6	MR
3326-1-1б	5/4	6.3	R	30/4	37.5	MR	5/4	7.1	R
3548-4-1б	0	0	VR	0	0	VR	10/4	14.3	R
4340-4-1	0	0	VR	5/2	2.5	VR	25/4	37.6	MR
01/165-91	5/4	6.3	R	5/4	6.3	R	10/4	14.3	R
S11/631	5/4	6.3	R	0	0	VR	25/4	37.6	MR
2026-1-811-97	0	0	VR	0	0	VR	25/4	37.6	MR
01/29-81-101	5/4	6.3	R	0	0	VR	15/4	21.4	R
01/29-94-1021-21	5/4	6.3	R	25/4	31.2	MR	0	0	VR
05N12-21	25/4	31.2	MR	30/4	37.5	MR	10/4	14.3	R
05N26-21	5/4	6.3	R	5/4	6.3	R	10/4	14.3	R
05N57-22	0	0	VR	25/4	31.2	MR	10/4	14.3	R
05N83-22	0	0	VR	0	0	VR	25/4	37.6	MR
06N139-22	0	0	VR	5/4	6.3	R	25/4	37.6	MR
06N153-23	30/4	37.5	MR	5/4	6.3	R	5/4	7.1	R
06N50-21	0	0	VR	5/4	6.3	R	10/4	14.3	R
M. amber	80/4	100	VS	80/4	100	VS	70/4	100	VS

On the whole, the investigated lines with race non-specific resistance constituted 45 % of all studied lines, the lines with field resistance of the *slow rusting* type – 43 %, and the lines with probable partial race specific resistance – 12 %. The percent distribution of the lines with a certain type of resistance is presented in Figure 1.

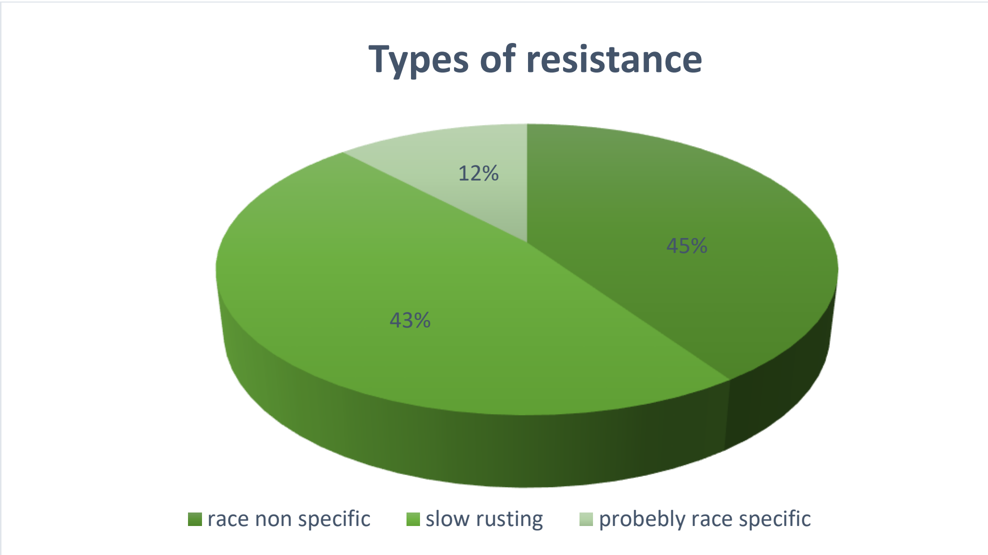

Figure 1. Percent distribution of the lines with certain type of resistance

Conclussion

As a result from the investigation carried out, the studied lines can be divided into three main groups:

- Lines, which responded at young stage with susceptible reaction to all pathotypes, or with resistance to one or two isolates, while in field demonstrating high resistance to resistance (VR – R). These were lines with expressed race non-specific resistance and constituted 45 % of the investigated lines.
- Lines, which responded with susceptibility at young stage, but their field resistance was within VR – MR. This type of resistance was also of race non-specific nature, but of the *slow rusting* type. This group of lines constituted 43 % of all studied lines.
- Lines, which at young stage responded with resistance to more than two pathotypes of *P. triticina*, and at adult stage their resistance was within VR – R. These were lines that could be carriers of partial race specific resistance in combination with race non-specific one. They accounted for 12 % of the investigated material.

The tested forty-two lines demonstrated a certain type of resistance, which can be considered efficient and they can be involved in the breeding programs for development of cultivars resistant to leaf rust.

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