



Original article

## Phytopathological Assessment of Lines of Common Winter Wheat to the Cause of Brown Leaf Rust (*Puccinia recondita f. sp. tritici*)

Zlatina Uhr <sup>a,\*</sup> & Evgeniy Dimitrov <sup>a</sup>

<sup>a</sup> Institute of Plant Genetic Resources „K. Malkov“ – Sadovo, Bulgaria

### Abstract

The study was conducted in the period 2017-2019 in the experimental fields of the Institute of Plant Genetic Resources “K. Malkov” in Sadovo, near Plovdiv, Bulgaria. At the infectious area, the immune responses of twenty-two genotypes of common winter wheat to the causative agent of brown leaf rust - *Puccinia recondita f. sp. Tritici* were tested. The line MX 289/2048 has shown high resistance to the pathogen, and seven samples are characterized by a stable reaction.

The biggest share of the studied breeding materials showed average resistance to the phytopathogen. A comparison of the indicators of grain weight in the central spike and 1000 kernel weight was made for samples grown on an infectious site and under natural field conditions. The obtained results showed an exceeding the values of the trait in favor of the samples grown under natural field conditions. It was found that the greatest influence on the traits grainsweight in the central spike and 1000 kernels weight has the year of cultivation. To assess the genetic diversity, a cluster analysis was applied, in which the selection materials were grouped into five main cluster groups. The lines MX 286/1777 and MX 270/3461 are characterized by the greatest genetic proximity, and the line MX 285/1058 is genetically the most distant compared to the samples from the first cluster group.

**Keywords:** Winter Common Wheat; Brown Leaf Rust; Immune Reactions; Type of Infection; Degree of Attack.

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\* **Corresponding author:**

Zlatina Uhr, Institute of Plant Genetic Resources „K. Malkov“ – Sadovo, Bulgaria.  
Email: [cosar.tosun@tkdk.gov.tr](mailto:cosar.tosun@tkdk.gov.tr)

## **INTRODUCTION**

Wheat is a main cereal crop associated with human nutrition. The needs for wheat grain are constantly growing, as the biggest share of the total harvested areas with cereals in 2017 is occupied by wheat 64% ([https://www.mzh.government.bg/media/filer\\_public/2019/05/22/agraren\\_doklad\\_2019\\_bg.pdf](https://www.mzh.government.bg/media/filer_public/2019/05/22/agraren_doklad_2019_bg.pdf)). The main goal of modern breeding programs is to look for sources to overcome the susceptibility of bread wheat to economically important diseases. In this regard, fungal phytopathogens are of great importance because they have a very harmful potential and in a short time can spread over large areas, and at the same time lead to severe damage to yield and quality of plant production. Wheat diseases of great economic importance include brown leaf rust, yellow rust and black rust (Bolton et al., 2008; Chen, 2005, Stoyanov, 2012;). To achieve quality disease resistance, it is necessary to use certain donors who have proven their high resistance to economically important pathogens (Iliev, 2009). The creating and growing sustainable varieties is the best strategy to control common fungal diseases in economically important cereal diseases. However, the use of varieties with resistance due to single genes is not completely effective for a longer period of time. The pathogens that cause these diseases are very dynamic and their races are constantly changing. The widespread use of the same disease-resistant varieties in recent years has led to the emergence of more virulent breeds, which has hampered breeders' efforts to create varieties with long-term resistance (Todorovska et al., 2009). The issue of resistance to biotic factors is being addressed by researchers in a new way. Tolerant varieties are considered to be safer for production. The world has created a rich assortment of resistant genetic plasma to the causes of brown, yellow and stem rust. Related wheat species have also been used as sources of resistance (Dimov et al., 1997; Browning, 1988; Singh & Rajaram, 1992; Karzhin et al., 1994;). The IPGR-Sadovo, GAI-G. Toshevo and Plant Protection Institute – Kostinbrot, Bulgaria / have collections of sustainable forms and complex resistance to the three species.

## **MATERIAL and METHODS**

The research was conducted in the period 2017-2019 in the infectious field of the Institute of Plant Genetic Resources "K. Malkov - the town of Sadovo, Plovdiv, Bulgaria The study included 21 newly selected lines of common winter wheat and variety Sadovo 1. The experiment was performed in blocks scheme. The sowing was performed according to the nest method used by Simson (1969) (circular sowing) and included a nest sown with the studied sample, followed by a nest sown with the Michigan amber variety, which is a standard for sensitivity.

Artificial inoculation was performed on the sensitive Michigan amber variety in the stem elongation start phase by injecting a pooled inoculum with a spore suspension of brown leaf rust. The type of infection and the degree of attack were used to determine the immune response of the studied materials to the causative agent of brown rust.

The type of infection was reported 10-12 days after the date of artificial inoculation and was reported on the following scale (Dimov, 1988):

I - immune, no signs of attack

HR - highly resistant - only chlorotic or necrotic spots are formed and the parasite does not sporol

R - resistant, small uredospores are formed on necrotic spots

MR - moderately resistant - the parasite forms medium-sized sores on green islets surrounded by necrotic or chlorotic rings

X - indeterminate reaction, heterogeneous type of infection. Uredospores of different sizes, different types of infection, scattered on one leaf

MS - moderately susceptible - medium-sized sores, without necrosis, sometimes weak chlorosis, surrounding the uredosors

S - highly susceptible, large uredosores are abundantly formed without necrosis and chlorosis

The degree of attack is expressed in percentages from 0.0% to 100.0% and reflects the leaf area occupied by the parasite. For easier comparability of the results, an average infection rate or the so-called corrected relative degree of attack (COSN)  $P_0$  has been calculated, modified in our country by Donchev (according to unpublished data) according to the formula:

$P_0 = X.K.100/X \text{ st.}$  Where: X- degree of attack of the test line (variety), X st.- degree of attack of the standard, K- coefficient depending on the type of infection: R-0.2; MR- 0.4; MS- 0.8; S- 1.00 The Michigan amber variety was used as a standard for susceptibility to the studied pathogen.

Depending on the obtained values of  $P_0$ , the tested lines and varieties are grouped into 5 categories according to the methodology of Mihova et al. (1990) : Group I-Highly resistant (HR)  $P_0=0-5\%$ ; Group II- resistant (R)  $P_0 = 6-25\%$ ; Group III-Moderately resistant (MR)  $P_0=26-45\%$ ; Group IV-Moderately susceptible (MS)  $P_0=46-65\%$ ; Group- Susceptible (S)  $P_0 =66-100\%$ .

To determine the impact of the disease, biometric measurements were made in three replicates of the infectious area and under natural field conditions (without artificial contamination) of the traits grainsweight in the central spike (g) and 1000 grainsweight (g). The statistical-mathematical processing of the experimentally obtained data from the elements of productivity was performed with the statistical program SPSS 18.0.

## RESULTS and DISCUSSION

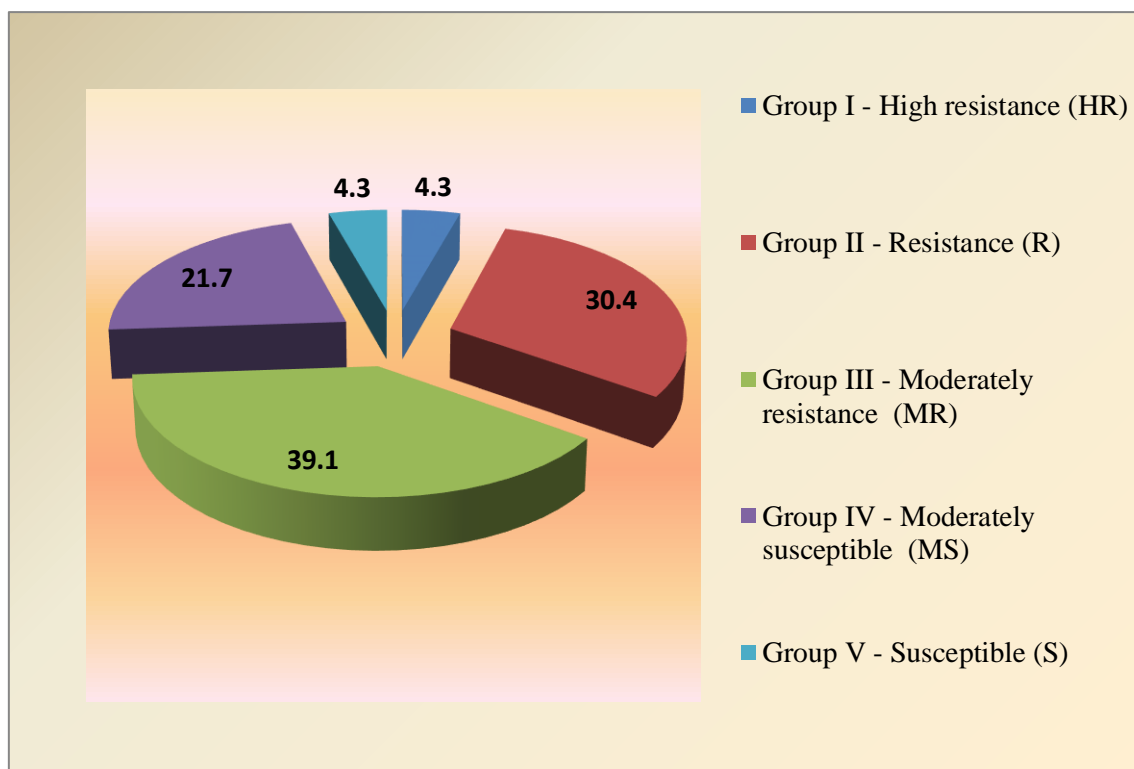
Table 1 presents a phytopathological evaluation of the studied breeding materials to the causative agent of brown leaf rust - *Puccinia recondita f. sp. tritici*. As a result of the calculated adjusted relative degree of infestation, the wheat genotypes were grouped according to their resistance. The obtained results show that the group of highly resistant specimens (group I) includes only line MX 289/2048, as

the degree of attack varies from 0 to 10.0%, and the type of infection is from resistant (2017, 2018) to immune (2019).

The group of resistant samples (group II) consists of seven lines of common wheat - RU 91/1748, MX 298/2622, MX 298/2580, MX 270/3464, MX 286/1777, MX 270/3461 and MX 270 / 27. The lowest degree of attack is characterized by line MX 298/2580, and the highest value for this indicator is reported for MX 270/3464 - 40.0%. By type of infection, the results in the table show that the selected breeding materials reacted with an immune to moderately sensitive type to the disease.

The group of moderately resistant wheat genotypes (group III) is the most numerous, constituting 39.1% of the total number of studied materials (Figure 1). Characteristic of this group is the higher rate of attack reaching 40.0% (MX 187/3) and the heterogeneous type of infection varying in different years from resistant to sensitive, and in the 2018 harvest moderately sensitive and susceptible type of infection is observed in all samples falling into this group.

The group of moderately susceptible selection materials (group IV) includes lines RU 47/3504, MX 270/3462, MX 270/3463, MX 285/1058 and variety Sadovo 1. The highest value (40.0%) of the indicator degree of an attack was reported at Sadovo 1 (2018), MX 270/3463 (2019) and MX 285/1058 (2019). All studied specimens falling into this group are characterized by a moderately susceptible and susceptible type of infection, with the only exception observed in line MX 270/3462, which reacted with a moderately resistant type in the growing season 2018.



**Figure 1.** Percentage distribution of the groups of immune manifestations

From the studied immune responses of the studied wheat genotypes to the causative agent of brown leaf rust, we can conclude that a large part of the breeding materials showed a different type of infection during the different years of the study. It was observed that the individual selection materials reacted with a resistant or moderately resistant type of infection, and in the second year of the study a moderately susceptible or susceptible type of infection was reported.

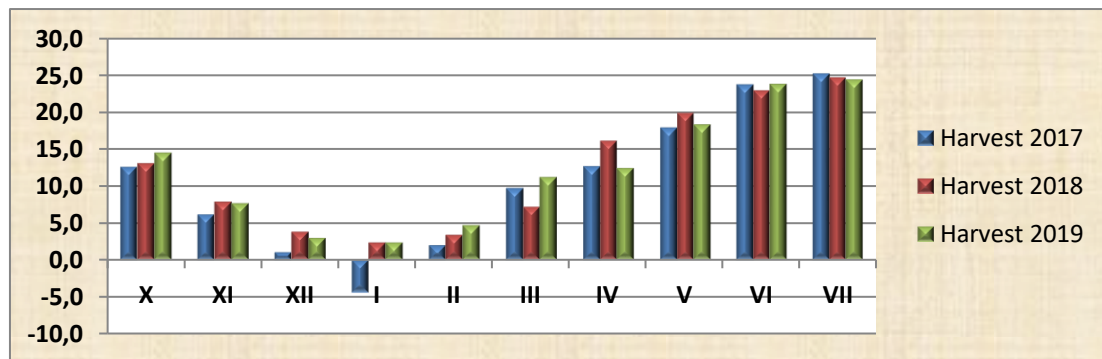
This is explained by the presence of various virulence genes, formed over the years in the population of the pathogen, which overcomes the protective mechanisms of the plant. This fact has been established by other authors (Donchev et al., 2009; Iliev, 2009). The different type of infection, ranging from resistant to susceptible, shows the possibility that these breeding materials are carriers of a vertical type of resistance, and this resistance is effective only against certain races of the pathogen.

This is most characteristic of the following wheat lines: MX 274/717 (type of infection - R, MS, S); MX 187/3 (type of infection - R, MS, MS); MX 265/3430 (type of infection - S, S, R); MX 286/1759 (type of infection - R, S, R) and others. With regard to horizontal resistance, which is effective against all races of the pathogen, only line MX 289/2048 has a low degree of attack ranging from 0 to 10%, as well as an immune and resistant type of infection to the disease. Horizontal stability in wheat has been reported by a number of other authors (Iliev, 2009; Nedyalkova et al., 2014; Kolmer & Oelke, 2006; Sadovaya et al., 2014)

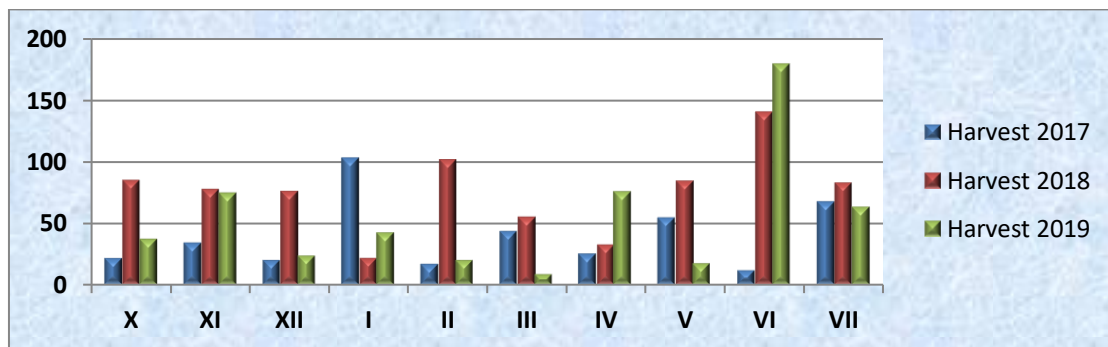
**Table 1.** Reaction of common winter wheat genotypes to the causative agent of brown leaf rust under the conditions of a field infectious area for the period 2017-2019

№	Line, variety	Degree of attack (%), type of infection	Po,%	Reaction to Po	Degree of attack (%), type of infection	Po,%	Reaction to Po	Degree of attack (%), type of infection	Po,%	Reaction to Po	P mean	Reaction to P mean.	Group
		2017			2018			2019					
1	MX 289/2048	10 R	5	HR	5 R	2	HR	0 I	0	HR	2.3	HR	I
2	RU 91/1748	20 MR	20	R	10 MR	8	R	15 MR	12	R	13.3	R	II
3	MX 298/2622	10 R	5	HR	5 R	2	HR	30 MS	48	MS	18.3	R	
4	MX 298/2580	0 I	0	HR	15 MR	12	R	35 MS	56	MS	22.7	R	
5	MX 270/3464	10 R	5	HR	40 MS	64	MS	5 R	2	HR	23.7	R	
6	MX 286/1777	10 R	5	HR	35 MS	56	MS	15 MR	12	R	24.3	R	
7	MX 270/3461	15 MS	30	MR	10 R	4	HR	25 MS	40	MR	24.7	R	
8	MX 270/27	25 MR	25	R	10 R	4	HR	30 MS	48	MS	25.7	R	
9	MX 286/1759	15 R	7.5	R	35 S	70	S	5 R	2	HR	26.5	MR	
10	MX 298/2582	20 R	10	R	20 MS	32	MR	30 MS	48	MS	30.0	MR	
11	RU 76/1332	30 MR	30	MR	35 MS	56	MS	5 MR	4	HR	30.0	MR	
12	RU 33/3244	25 MS	50	MS	35 MS	56	MS	10 R	4	HR	36.7	MR	
13	MX 265/3430	20 S	50	MS	30 S	60	MS	10 R	4	HR	38.0	MR	
14	MX 274/717	5R	2.5	HR	20 MS	32	MR	40 S	80	S	38.2	MR	
15	MX 295/2524	25 MS	50	MS	35 MS	56	MS	15 MR	12	R	39.3	MR	
16	RU 91/1729	30 MR	30	MR	30 MS	48	MS	30 MS	48	MS	42.0	MR	
17	MX 187/3	15 R	7.5	R	40 MS	64	MS	35 MS	56	MS	42.5	MR	
18	RU 47/3504	25 MS	50	MS	30 MS	48	MS	25 MS	40	MR	46.0	MS	IV
19	MX 270/3462	30 S	75	S	20 MR	16	R	25 S	50	MS	47.0	MS	
20	MX 270/3463	20 MS	40	MR	30 MS	48	MS	40 MS	64	MS	50.7	MS	
21	MX 285/1058	20 MS	40	MR	30 MS	48	MS	40 MS	64	MS	50.7	MS	
22	Sadovo 1	15 MS	30	MR	40 S	80	S	35 MS	56	MS	55.3	MS	
23	Michigan Amber-st	40 S	100	S	50 S	100	S	50 S	100	S	100.0	S	V

Another characteristic feature influencing the variation of the signs, the degree of attack and the type of infection are the meteorological conditions during the specific study period. Warm days (20-25°C) and nights with showers contribute to the mass development of brown rust, especially in the afternoon and the formation of dew. The development of the disease is especially intense in wet autumn, relatively mild winter and in wet and rainy weather in the period of emergence-flowering (Stavcheva, 2003; Prigge et al., 2004). Figure 2 and Figure 3 show the average monthly temperatures and the sum of precipitation for the study period.



**Figure 2.** Average monthly air temperature for the study period, °C



**Figure 3.** Sum of precipitation for the study period, mm

Observing the development of brown rust in individual years in connection with agro-climatic conditions, we can conclude that in 2017 the lowest rate of attack (0-40%) on the studied breeding materials was reported compared to the results obtained in 2018 (5-50 %) and 2019 (0-50%) years. The results are similar for the indicator type of infection, as in 2017 the least samples reacted with a moderately sensitive and sensitive reaction to the disease (9 pcs.), and their number is higher in 2018 (16 pcs. ) and 2019 (14 pcs.).

As a reason for this development of the disease we can point to the higher air temperatures in October, November, December and May 2018-2019 compared to 2017, as well as the higher amount of precipitation in the fall of 2018 and 2019 years. During these years, a relatively high amount of

precipitation is observed during the emergence of wheat, contributing to the greater multiplication of brown rust.

To determine the harmful effect of leaf rust on the studied samples, biometric measurements of the traits mass of grains of central class and mass of 1000 grains from the infectious area and under natural field conditions were made (Table 2). The results of the table show that in the indicator grain size in the central class the genotypes grown under natural conditions showed better results compared to the genotypes grown on an infectious site. The lines RU 76/1332 (3.1%), MX 289/2048 (3.4%) and MX 298/2582 (3.5%) have the smallest difference between them, and the highest loss was reported for line RU 33 / 3244 - 39.0%.

The results obtained with line MX 270/3462 are also impressive, as it has reacted with a moderately sensitive reaction to brown rust and at the same time the reported loss in grain mass is relatively low (16.4%). For this reason, we can say that the mentioned sample is a carrier of the tolerance quality valuable for immunoselection.

By the indicator 1000 kernel weight, the obtained results show us again excesses of the holiday in the genotypes grown on natural field conditions in comparison with the breeding materials from the infectious area. The only exception is observed in line MX 298/2622, which showed a better result on an infectious background. With 54.4% of the total number of breeding materials, the reported loss of the trait is in the range between 10 and 20%.

The greatest harmful effect of brown rust on the 1000 kernel weight was reported in lines RU 91/1748 (31.1%), RU 47/3504 (23.8%) and MX 270/3464 (20.6%), and the least affected by The pathogens are samples MX 298/2622 (-2.3%) and MX 285/1058 (3.2%), and for the last mentioned line we can say that it is tolerant to the disease as it is characterized by low loss in the 1000 kernel weight and at the same time is showed moderate susceptibility to the pathogen



**Table 2.** Average values of the indicators grains weight in central spike, g and 1000 kernel weight, g from the infectious area and field conditions (2017-2019)

№	Genotype	Grains weight in central spike, g			1000 kernel weight, g		
		Infectious area - Experiment 1	Fields conditions - Experiment 2	Difference,%	Infectious area - Experiment 1	Fields conditions - Experiment 2	Difference,%
1	MX 289/2048	2.27	2.34	3.4	43.8	45.5	3.9
2	RU 91/1748	1.90	2.09	9.9	34.6	45.4	31.1
3	MX 298/2622	1.77	2.04	15.7	37.4	36.5	-2.3
4	MX 298/2580	1.48	1.97	33.1	33.9	38.1	12.4
5	MX 270/3464	1.82	2.04	12.2	38.5	46.4	20.6
6	MX 286/1777	2.13	2.32	8.9	40.1	45.7	13.8
7	MX 270/3461	1.78	1.94	9.4	41.0	46.7	13.9
8	MX 270/27	1.59	1.77	11.2	37.8	45.4	20.2
9	MX 286/1759	1.83	2.17	18.2	37.3	42.7	14.4
10	MX 298/2582	1.92	1.99	3.5	36.6	41.6	13.9
11	RU 76/1332	1.81	1.87	3.1	41.5	47.7	15.0
12	RU 33/3244	1.37	1.90	39.0	42.3	47.5	12.2
13	MX 265/3430	1.79	2.19	22.4	38.2	43.1	12.7
14	MX 274/717	1.57	1.83	17.0	42.5	46.4	9.1
15	MX 295/2524	1.67	2.07	24.0	39.9	46.6	16.8
16	RU 91/1729	1.59	1.80	13.3	39.1	44.4	13.5
17	MX 187/3	1.70	2.07	21.6	34.6	37.4	8.1
18	RU 47/3504	1.68	2.08	23.8	33.5	41.5	23.8
19	MX 270/3462	1.69	1.97	16.4	40.9	47.3	15.7
20	MX 270/3463	1.82	2.13	17.1	38.1	45.2	18.6
21	MX 285/1058	1.43	1.82	27.1	35.8	36.6	2.3
22	Sadovo 1	1.78	2.09	17.4	38.4	45.5	18.7

A multivariate analysis of variance was applied to determine whether the variability of grain weight of central spike and 1000 kernel weight traits depended more on genetic factors, the influence of brown rust, or growing conditions. It assessed the strength of the influence of the sources of variation - genotype, disease, environment and their interactions. The results in Table 3 show that the strongest influence on the grain weight of central spike has the growing conditions ( $\eta = 30.5\%$ ) followed by the interaction of genotype factors with the environment ( $\eta = 22.2\%$ ).

The influence of the disease factor x environment ( $\eta = 0.1\%$ ) is the weakest and not proven. On the 1000 kernel weight again growing conditions ( $\eta = 32.0\%$ ) had a primary importance on the indicator, and in second place is the genotype of plants ( $\eta = 25.2\%$ ). With this feature, the influence between the

factors disease and environment is the weakest ( $\eta = 0.6\%$ ). The influence of brown rust on the grains weight in the central spike and and 1000 kernel weight is relatively low and has values of  $\eta$ , respectively 7.6 and 10.3%.

**Table 3.** Analysis of the variance – ANOVA

Elements of productivity	Sources of variation	SS	df	MS	F exp.	F tab.	$\eta$ , %
Grains weight in central spike, g	Genotype: factor A	11.3	21	0.5	42.7***	2.3	11.3
	Disease: factor B	7.6	1	7.6	606.4***	11.1	7.6
	Environment: factor C	30.5	2	15.2	1213.4***	7.1	30.5
	Interaction: AxB	1.6	21	0.1	6.1***	2.4	1.6
	Interaction : AxC	22.2	42	0.5	42.1***	1.9	22.2
	Interaction : BxC	0.1	2	0.03	2.1 n.s.	3.0	0.1
	Interaction: AxBxC	3.2	42	0.1	6.1***	1.9	3.2
	Error	3.3	264	0.0			3.3
	Total	79.7	395				100
1000 kernel weight, g	Genotype: factor A	4739.5	21	225.7	51.4***	2.3	25.2
	Disease: factor B	1936.0	1	1936.0	440.7***	11.1	10.3
	Environment: factor C	6007.1	2	3003.5	683.6***	7.1	32.0
	Interaction: AxB	720.0	21	34.3	7.8***	2.4	3.8
	Interaction : AxC	2751.2	42	65.5	14.9***	1.9	14.7
	Interaction : BxC	114.2	2	57.1	13.0***	7.1	0.6
	Interaction: AxBxC	1347.6	42	32.1	7.3***	1.9	7.2
	Error	1159.9	264	4.4			6.2
	Total	18775.6	395				100

SS - sum of squares; gf - degrees of freedom; MS - variance; F exp. - F experimental; F tab. - F tabular;  $\eta$  - force of influence of the factor (%); \*\*\* - proven at  $\alpha = 0.001$ , n.s.- unproven

To establish the genetic similarity between the studied breeding materials, a cluster analysis was applied. Cluster analysis is a method of classification and hierarchy in which the studied population is divided into a number of groups called clusters. The study of breeding materials through cluster analysis allows breeders to plan and make more effective decisions for the development of their breeding programs.

With the help of clustering, the samples can be divided by genotype, depending on its phenotypic manifestation by a certain trait or group of traits. The results of the clustering are presented in a dendrogram (Figure 4). The grouping of the lines was made on the basis of their resistance to brown rust, as well as from the results obtained from the grains weight in the central spike and the 1000 kernel weight from the infectious area and under field conditions.

The dotted horizontal line of the dendrogram shows the rescaled distance at which the clusters were formed. From the presented figure it can be seen that the selection materials are grouped into five main cluster groups. The first group includes seven samples, which are grouped into two subgroups (I subgroup: MX 286/1777, MX 270/3461, MX 270/3464, MX 270/27; II subgroup: RU 76/1332, MX 286/1759 and MX 298/2582).

Line MX 286/1777 and line MX 270/3461 are characterized by the greatest genetic similarity. Characteristic of this group is that all selection materials from the first subgroup reacted with a stable immune response to brown rust, and a relatively low loss in grain mass of the central class (8.9-12.2%) between the two experiments. The samples from the second subgroup are characterized by average resistance to brown rust, as well as a minimal difference in the mass of 1000 grains from the two experiments, ranging from 13.9 to 15.0%. Some of the materials in the first group (MX 270/3461, MX 270/3464, MX 270/27) also come from a cross - Albena x Diamand.

A second cluster group is represented by four wheat samples - MX 298/2622, MX 298/2580, RU 91/1748 and MX 289/2048. With the exception of line MX 289/2048 showed high resistance to the disease, all other lines in this group have a stable immune response to the pathogen. The lines MX 298/2622 and MX 298/2580 form a separate fork and originate from a cross - Enola x Guinness.

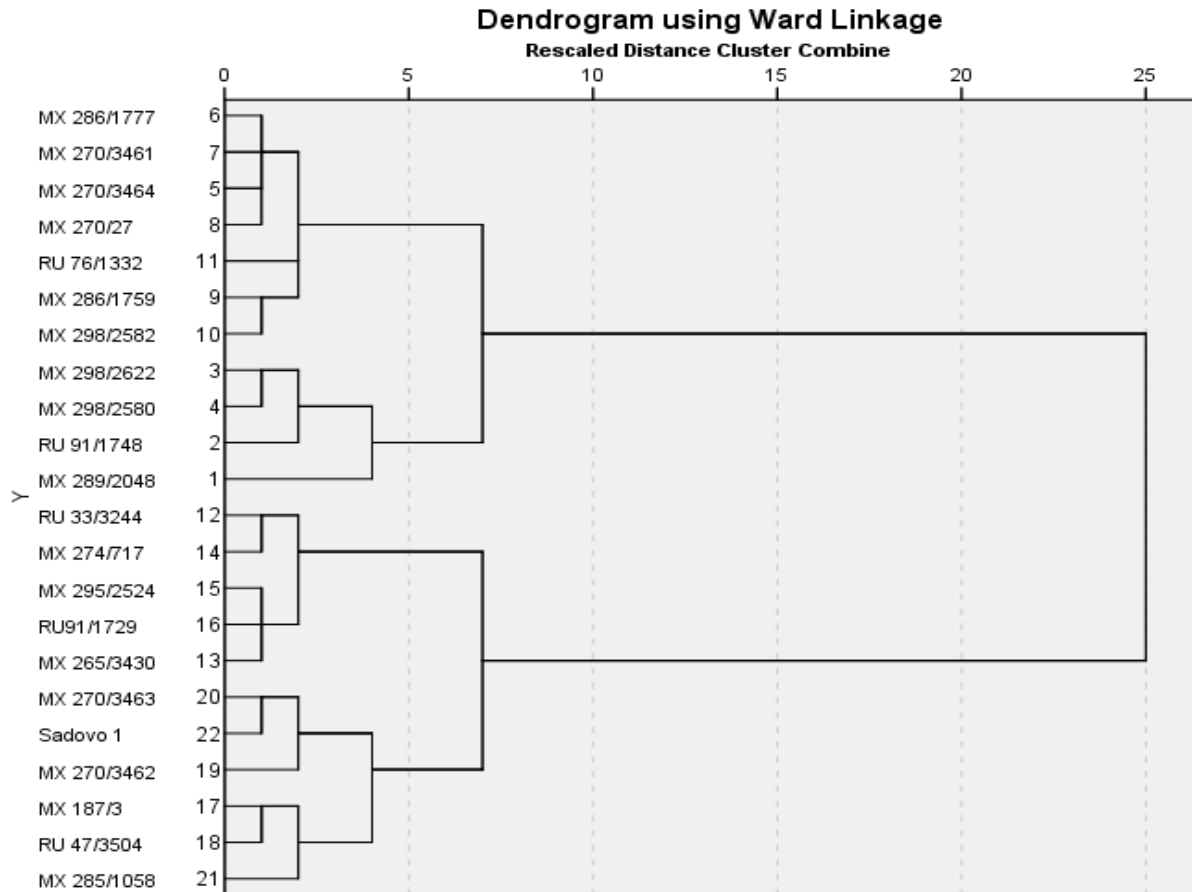
A third cluster group is divided into two subgroups, the first subgroup consisting of lines PU 33/3244 and MX 274/717, and the second subgroup includes samples MX 295/2524, RU 91/1729 and MX 265/3430. All representatives forming this group are characterized by average resistance to leaf rust and close in value results from the indicator 1000 kernel weight.

The fourth cluster group includes the variety Sadovo 1, as well as the lines MX 270/3463 and MX 270/3462, belonging to the parent pair Fermer x Prelom. The samples from this group belong to the moderately sensitive wheat genotypes, and they have minimal differences in the values of the indicators of the grains weight in the central spike and 1000 kernel weight from the infectious area and under natural field conditions.

The fifth cluster group consists of the lines MX 187/3, RU 47/3504 and MX 285/1058, as the characteristic of the group is its heterogeneity in comparison with the other cluster groups. In terms of resistance to brown rust, line MX 187/3 showed moderate resistance, and the other two lines showed moderate susceptibility to the pathogen.

Of note is the fact that in the indicator 1000 kernel weight the samples MX 285/1058 and MX 187/3 have an insignificant difference between the two experiments, respectively 2.3 and 8.1%, and in line RU 47/3504 the loss is 23.8%. Regarding the genetic distance depending on the rescaled distance at which the cluster groups are formed, the dendrogram shows that the line MX 285/1058 is genetically the furthest from the selection materials falling into the first cluster group

From the presented cluster analysis we can summarize that the main criteria by which the samples are grouped into separate cluster groups is based on their resistance to brown leaf rust. According to a number of authors (Fang et al., 1996; Khodadadi et al., 2011; Siahbidi et al., 2013), it can be generally accepted that division by cluster analysis gives the best assessment of the genetic remoteness of genotypes and therefore, the use of cluster analysis is preferable in the study of genetic diversity



**Figure 4.** Dendrogram of cluster analysis

### Conclusions

High resistance to brown leaf rust was found in line MX 289/2048, and pathogen resistance was found in seven genotypes of winter wheat.

Samples that have shown a highly resistant and persistent immune response can be successfully used in immunoselection as sources of starting material for the creation of new varieties with increased resistance to brown rust

The genotypes of common winter wheat tested in natural field conditions are superior to the genotypes of common winter wheat tested on an infectious site in terms of grain weight of the central spike and 1000 kernel weight.

The strongest and most proven is the influence of the environment on the indicators of grains weight of central spike and of 1000 grainsweight

The greatest genetic similarity is between lines MX 286/1777 and MX 270/3461, and genetically the most distant is line MX 285/1058 compared to the samples from the first cluster group.

Genetically distant materials belonging to different cluster groups can be used in the selection process as sources for achieving genetic diversity in the selection of common winter wheat.

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