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REACTION OF RYE CULTIVARS TO LEAF RUST (*P. recondita* f. sp. *secalis*) IN THE CONTEXT OF CLIMATE CHANGE IN DRY AREA IN SOUTHERN ROMANIA

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Abstract

*Increasing temperatures and changing precipitation patterns impact plants biotic constrainers worldwide affecting host-pathogen relationship depending on geographical and temporal distribution of inoculum amount and cultivars susceptibility. Leaf rust of rye, which is caused by the obligate biotrophic basidiomycete Puccinia recondite f. sp. secalis (Roberge ex. Desmaz) has become one of the most important limiting factors for rye production in Central and Eastern Europe. During 2019-2020 growing season, a plant–pathogen interaction profile was observed on four rye genotypes in a randomized complete block design with three replications in dry area from Research and Development Station for Plant Culture on Sands Dăbuleni in south of Romania. Adult plant partial resistance was assessed through host response and epidemiological parameters as final rust severity (FRS), area under the disease progress curve (AUDPC), relative area under the disease progress curve (rAUDPC), coefficient of infection (CI) and infection rate (IR). The response of rye genotypes to leaf rust included different variation in resistance reaction ranging from moderately resistant (Serafino, Bintto), moderately susceptible (Inspector) and very susceptible (Suceveana). A negative and highly significant correlation of AUDPC with grain yield (r =-0.9194***) was found during 2019-2020 cropping season.*

***Key words**: epidemiological parameters, leaf rust, adult plant partial resistance, Puccinia recondite f. sp. secalis.*

INTRODUCTION

During the last decades global food production and food security increased significantly due to many changes in agricultural systems as a consequence of interaction among multiple factors, such as world population increase, urbanization, technical progress, income growth, genetically progress, improved cropping technologies, globalization on food production, machinery revolution, markets, consumption, faster access to the information (Matei, 2011; Matei and Roșculete, 2011; Partal et al., 2013; Partal et al., 2014; Cristea et al., 2015; Bonciu, 2018, Roșculete and Roșculete, 2018; Bonciu, 2019, Roșculete et al., 2019; Bonciu, 2020; Partal and Paraschivu, 2020).

However, this progress comes to face additional factors as climate variability and climatechanges. Worldwide the cultivation of crops, their yielding capacity and quality are directly affected by different climatic factors or extreme climatic events.

European agricultural systems are also affected by climate change through changes in temperatures (warmer than long-term means or unseasonal frosts) and precipitation (including snow, hail or extreme intensity, variable humidity) impacting directly crops production and availability and indirectly the biotic constrainers of crops, such as weeds, pests and pathogens and their relationship with plants (Coakley et al., 1999; Downing et al., 2000; Chakraborty and Pangga, 2004; Eastburn et al., 2011; Ză1ă et al., 2012; Cotuna et al., 2013; Paraschivu et al., 2015; Paraschivu et al., 2014; Paraschivu et al., 2017; Cotuna et al., 2018; Juroszek et al., 2019; Paraschivu et al., 2019a; Paraschivu et al., 2019b; Casazza et al., 2021).

Climate change together with human-induced changes is expected to cause the spread of pathogens, pests and invasive species in areas where they have not been relevant before, bringing new challenges for crop management and breeding in order to face yield losses and avoid alteration of natural landscape vegetation (EEA Report, 2017). Thus, some pathogens tend to become more aggressive even in cropping systems based on crops diversifycation by minor cereals.

Rye (*Secale cereale*) is a minor cereal, closely related to barley and wheat, used for human consumption as rye bread and alcoholic beverages, such as beer, whiskey and vodka. Also, rye is very important feed for livestock and currently contributes to crop species diversity in temperate regions of Central and Eastern Europe, especially in marginal environments where soil and climate are unfavourable for wheat production. In 2020 European Union (EU) produced 9,175,000 tons of rye grains from which 71.82% was produced in Germany and Poland (USDA, 2020).

One of the most important disease of rye in Central and Eastern Europe is Brown rust (BR), known also as Leaf rust (LR), caused by the obligate biotrophic basidiomycete *P. recondita* f. sp. *secalis* (Prs) (Roberge ex.Desmaz) (Roux et al., 2007; Roux and Wehling, 2010; Meidaner et al., 2012). Yield losses can be up to 40% in natural conditions, but they can be as high as 80% in case of early infection (Solodukhina, 2002; Wehling et al., 2003). This mainly happens to the pathogen`s ability to multiply rapidly, as well as to its air borne dispersal mechanism from one field to another (Brownand Hovmøller, 2002).

Considering important losses due to Leaf rust, genetic resistance is currently considered as the most economical and effective control measure of this disease, causing no additional cost to the farmers and reducing the use of fungicides. (Singh et al., 2005). However, cereal rusts exhibit considerable capacity for generating, recombining and selecting for resistance under the impact of climate variability and they can adapt to new environment, despite the fact that currently we are not able to predict accurately the trajectory of each pathosystem under climate change. Therefore, screening rye cultivars or using the marker-assisted selection in rye breeding program is of great importance to find new resistance genes associated with leaf rust resistance.

In this context the present paper emphasises the results of the assessment of four rye genotypes, with different origins, screened for adult plant partial resistance to *P. recondita* f. sp. *secalis* in natural infections in the sandy soils in Southern Oltenia, Romania

MATERIALS AND METHODS

The trail for screening different rye genotypes for their adult plant partial resistance to *P. recondita* f. sp. *secalis* was carried out during 2019-2020 growing season at the Development Research Station for Plant Culture on Sands Dabuleni, located in Southern Oltenia, Romania (43°48′04″N 24°05′31″E), on sandy soil, poorly supplied with nitrogen (between 0.04-0.06%), well supplied with phosphorus (between 54 ppm and 77 ppm), reduced to a medium supplied with potassium (between 64 ppm to 83 ppm), low in organic carbon (between 0.12-0.48%) and weakly acidic pH to neutral (between 5.6 and 6.93).

Technological measures applied included broadcasting the fertilizers at sowing time with $N_{80}P_{80}K_{80}$, one side nitrogen fertilization during vegetation with N_{70}, starter irrigation with 250 m^3 water/ha and supplemental irrigation with 300 m^3 water/ha at heading stage. Also, weeds control was done using Dicopur Top 464 SL (1 l/ha) applied in postemergence to control annual and perennial dicotyledons accordingly with the recommendations (cereals to the formation of the first internode and the weed species in the small phase of about 2-4 leaves and a maximum of 10-15 cm high for perennial weeds).

A plant-pathogen interaction profile was observed on four rye genotypes (Serafino, Bintto, Inspector and Suceveana), assessed for their response to natural infection with *P. recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) in a randomized complete block design (RCBD) with three replications. Each plot had 5 m^2, a space of 1 m between blocks and 0.5 m between plots.

Disease observations were recorded since the first appearance of leaf rust infection on the susceptible rye genotypes until rust symptoms were fully developed (nearly at the early dough stage). Adult plant partial resistance for leaf rust was assessed through host response and epidemiological parameters as final rust severity (FRS), area under the disease progress curve (AUDPC), relative area under the disease progress curve (rAUDPC), coefficient of infection (CI) and infection rate (IR).

Rye genotypes response was expressed in five infection types for cereals leaf rust according to Johnston and Browder (1966) (Table 1).

Table 1. Infection types of cereals leaf rust used in disease assessment at seedling stage adopted by Johnston and Browder (1966)

Infection type	Host response	Symptoms
0	Immune	No uredia or other macroscopic sign of infection
0	Nearly Immune	No uredia, but hypersensitive necrotic or chlorotic flecks present
1	Very resistant	Small uredia surrounded by necrosis
2	Moderately resistant	Small to medium uredia surrounded by chlorosis or necrosis
3	Moderately susceptible	Medium-sized uredia that may be associated with chlorosis
4	Very susceptible	Large uredia without chlorosis or necrosis
X	Heterogenous	Random distribution of variable-sized uredia on single leaf

Leaf rust severity (%) was recorded for each genotype from the time of rust first pustules appearance (booting stage) until the early dough stage (Zadoks scale) (Zadoks et al., 1974), assessing 10 tillers randomly selected and pre-tagged plants of the central four rows of each plot and the mean of the ten plants was considered as the value for a plot. Rust severity was determined by visual observation and expressed as percentage coverage of leaves with rust pustules (from 1% to 100%) following Cobb's scale modified by Peterson (Peterson et al., 1948) (Table 2).

Table 2. Leaf rust severity expressed as percentage coverage of leaves with rust pustules - Cobb`s scale modified by Peterson (Peterson et al., 1948)

Category	Percentage leaf rust infection relative to susceptible check	Type of resistance
1	80-100%	Susceptible
2	50-70%	Race-nonspecific, low resistance
3	30-50%	Race-nonspecific, moderate resistance
4	10-20%	Race-specific, high resistance
5	less than 10%	Race-specific, high resistance
6	less than 5%	Effective, race-specific resistance

Final rust severity values were used to calculate Area under Disease Progress Curve (AUDPC), which shows the evolution and disease quantity on each rye genotype included in the trail, following the formula (Campbell and Madden, 1990):

$$\text{AUDPC} = \sum_{i=1}^{a}\left[\left\{\frac{Yi + Y(i+1)}{2}\right\} x(t(i+1) - ti)\right]$$

where, Yi = disease severity (%) at each measurement; ti = time in days of each measurement; a = number of Leaf Rust assessments.

Relative Area Under the Disease Progress Curve (rAUDPC) was calculated using the following formula:

rAUDPC = [AUDPC check/AUDPC assessed genotype] × 100

Average coefficient of infection (CI) was calculated by multiplying the percentage of disease severity and the constant value assigned to each infection type (Saari and Wilcoxson, 1974; Pathan and Park, 2006). The constant values were considered as R=0.2, R-MR = 0.3, MR = 0.4, MS = 0.8 and S = 1.

Apparent infection rate (IR) as a function of time was also calculated from the two disease severity observations as a severity of leaf rust infection at the time of rust pustules appearance and every fifteen days thereafter. It was estimated using the following formula adopted by Van der Plank (1963).

Inf-rate (IR) = 1/t (ln x/1-x)

Where x = the percent of disease severity divided by 100; t = time measured in days. The apparent infection rate is the regression coefficient of ln x/1-x on t.

In order to characterize the evolution of climatic parameters (air temperature, rainfall, humidity, wind speed) into the experimental field it was used an automatic weather station (AWS).

Means were compared with the susceptible genotype Suceveana (control). The results were statistically analysed and interpreted using the analyse of variance and mathematical functions of MS Office Excel 2010 facilities.

RESULTS AND DISCUSSIONS

The challenges to achieve sustainable food security in dry areas meet the ones generated by the effects of climate change and climate variability on crops health, especially in vulnerable crop systems like cereals, associated by many authors with changes in pathogens life cycles, increased incidence, pathogenicity, genetically recombination and aggressiveness traits (Chakraborty and Newton, 2011; Newton et al., 2011; West et al., 2012; Chakraborty, 2013; Elad and Pertot, 2014; Fones et al., 2020; Wolfe and Ceccarelli, 2020).

The 2019-2020 cropping season was favourable to rye Leaf rust disease in the dry area in Southern Oltenia, Romania. For scouting optimization and to predict the Leaf rust disease development, rainfalls and temperatures were taken into account. Humidity was determined by the amount of rain of 383.96 mm, comparatively with multiannual average rainfall of 376.85 mm, while the monthly average temperature was 13.9ºC comparatively with multiannual average temperature of 12.7ºC (Figure 1).

Figure 1. Climatic conditions during the study period (2020 year)

During January to August 2020 the monthly average temperature increased up to +1.2ºC comparatively with multiannual average temperature for January to August between 1956-2019 for the same geographic area. This temperature increase follows the global trend in planet warming. Thus, accordingly with a report of National Oceanic and Atmospheric Administration (NOAA, September 2020) monthly average temperature for January to August 2020 increased up to +1.03ºC (+15.03ºC) at the global level comparatively with average temperature recorded on Earth in the 20th century (+14ºC).

Rainfall amount for evaluated period was slightly higher with 7,11 mm than multiannual amount for dry areas in Southern Romania. The humidity at leaf level cumulated with increased temperature favoured the development of Leaf rust disease, which exhibited the first symptoms at the end of April 2020 on the 4th and the 3th rye genotypes leaves.

Optimal environmental conditions for disease development are temperatures ranging from 15°C to 20°C, but the fungus can develop at the temperature of 2-35°C. The fungus needs approximately six hours of moisture on leaves to start developing. With much moisture and suitable temperatures, lesions are formed within 7-10 days and spore production reduplicate another uredospore generation (Kolmer, 2013).

Săvulescu (1953) showed that uredospores of leaf rust were visible on rye leaves at the end of May or the beginning of June, but the currently results show that in the context of climate change, with higher monthly average temperature and ununiform rainfalls, these fruiting bodies of the pathogen (uredinia with uredospores) appear earlier. These findings suggest a modification of life cycle of the pathogen *P. recondita* f. sp. *secalis* by many generation numbers and higher resistance of uredospores to increased temperature. Also, Harvell et al. (2002) suggested that rising temperatures will (i) increase pathogen development transmission, and generation number; (ii) increase overwinter survival and reduce growth restrictions during this period and (iii) alter host susceptibility.

P. recondita f. sp. *secalis* spores are spread by splashing water and wind leading to many successive infections. Meidaner (2012) showed that minimum wind speed for uredospores splashing is 2m/s. In the experimental field the wind speed ranged between 5-40 km/h, respectively 1.4-14 m/s (Table 3).

Table 3. Wind speed km/h recorded during April-June 2020 in the experimental field*

Time	April	May	June
the 1st decade	11-35 km/h	14-40 km/h	-
the 2nd decade	14-34 km/h	5-37 km/h	-
the 3th decade	19-34 km/h	21-37 km/h	-

*automatic weather station DRSPCS Dabuleni, Romania

Identification of the fungus *P. recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) and its characteristics were done in the Phytopatology Laboratory of Agriculture Faculty in University of Craiova, using MOTIC microscope. The diameter of uredinia can reach even 1.5 mm, their colour is orange to brown and their shape is round to ovoid. The average size of uredospores release from uredinia is 20 mm in diameter and colour orange-brown (Figure 2). Uredospores have up to eight germ pores scattered in dense walls.

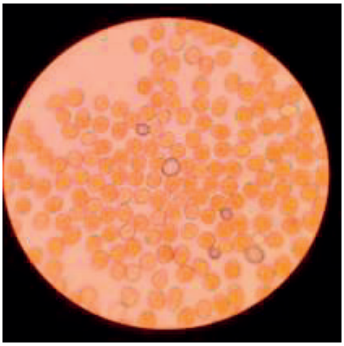

Figure 2. Uredospores of *Puccinia recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) (original photo Paraschivu Mirela, 2020)

Leaf rust pustules are small, circular to oval shape, with orange to light brown dusty spores (uredospores) on upper surface of leaves surrounded by a light-coloured halo (Figure 3).

Figure 3. Pustules with uredospores of *Puccinia recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) (original photo Paraschivu Mirela, 2020)

There can be thousands of spores in each pustule. In case of severe attack leaf rust pustules may extend also on the leaf sheaths, stalks and husks.

Following field screening, the response of rye genotypes to leaf rust included different variation in resistance reaction ranging from moderately resistant (Serafino, Bintto), moderately susceptible (Inspector) and very susceptible (Suceveana-control). These findings were also emphasised by partial resistance traits.

Adult plant data revealed that partial resistance traits (FRS, AUDPC, rAUDPC,CI and IR)showed a discrepancy in the values within parameters and genotypes (Table 4).

Table 4. Partial resistance traits to leaf rust in adult plant of four rye genotypes

Genotype	FRS	AUDPC	rAUDPC	CI	IR
Binnto	55.63**	113.75*	234.72**	22.25**	0.0373**
Serafino	43.86**	92.10**	289.90**	17.54**	0.0524**
Inspector	68.45	163.8	163.00*	54.76	0.0240
Suceveana	87.68	267.00	100	87.68	0.0083

FRS = Final Rust Severity; AUDPC = Area under disease progress curve; rAUDPC = Relative area under disease progress curve; CI = Coefficient of infection; IR = Infection rate.
**Significance level at $P \leq 0.01$
Suceveana = control

Thus, comparatively with Suceveana genotype (control), only Serafino and Binnto possessed high level of adult plant partial resistance based on the assessed traits, during 2019-2020 cropping season. Serafino recorded the lowest Final Rust Severity (FRS) (43.86%), which corresponds with low AUDPC value (92.10) and low Infection Coefficient (IC) (17.54). The differences for all resistance traits for Serafino and Binnto genotypes were highly significant comparatively with the control genotype.

Among all adult plant partial resistance traits was noticed a highly significant correlation (Table 5).

Table 5. Correlation coefficients (r)* for disease parameters of leaf rust on rye genotypes at DRSPCS Dabuleni during 2019-2020 cropping season

Disease parameter	FRS	AUDPC	rAUDPC	CI	IR
FRS	1	0.982***	-0.993***	0.979***	-0.994***
AUDPC		1	-0.957***	0.987***	-0.955***
rAUDPC			1	-0.972***	0.997***
CI				1	-0.960***
IR					1

FRS = Final Rust Severity; AUDPC = Area under disease progress curve; rAUDPC = Relative area under disease progress curve; CI = Coefficient of infection; IR = Infection rate.
* Pearsons' r_{calc}values

Negative high correlations were observed between Infection rate (IR) and FRS, AUDPC and CI in 2019-2020 cropping season. These findings indicate that although FRS, AUDPC and CI increased, the rate of infection (IR) reduced as epidemic progressed because less healthy plant tissue was available for additional infections.

The response of rye genotypes along with grain yield (t/ha) indicated the presence of inverse relation between the disease level (AUDPC) and grain yield. The highest significant loss percentages were found in susceptible genotypes Suceveana and Inspector. The value of determination coefficient (R^2 = 0.8453), for all rye genotypes assessed, indicated that up to 84% of variation in rye yield could be explained by AUDPC variability. It was noticed a highly significant correlation between AUDPC values and grain yield (r = -0.9194***) (Figure 4).

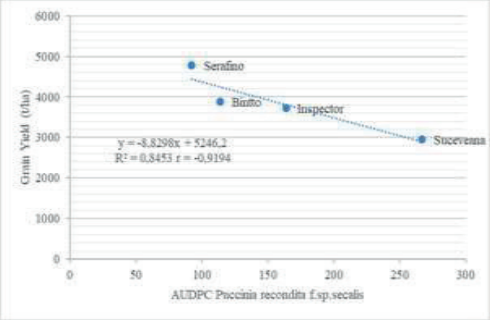

Figure 4. Relationship between Leaf rust AUDPC value and rye grain yield in 2019-2020 cropping season

Yield loses due to Leaf rust in rye in Europe were also reported previously by different authors (Solodukhina, 2002; Roux and Wehling, 2010; Meidaner et al., 2012).

However, the results of the experiment show that in the context of climate change the Leaf Rust is a serious disease of rye in dry marginal areas from Romania and climate variability can lead to further epidemics.

CONCLUSIONS

The present study was carried out to assess the adult plant response of four ryegenotypes to the attack of *P. recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) in natural infections in dry area from Southern Romania during 2019-2020 cropping season. The study emphasized that the increase of monthly temperature with +1ºC may lead to earlier incidence of the disease starting even with the end of April.

The response of rye genotypes to the Leaf Rust (LR) included different variation in plants reaction ranging from moderately resistant (Serafino, Bintto) to moderately susceptible (Inspector) and very susceptible (Suceveana), depending on genetic background and environmental conditions.

The values of Area under Disease Progress Curve (AUDPC) ranged from 92.10 (Serafino) to 267 (Suceveana). Serafino recorded the lowest Final rust severity (FRS) (43.86%), which corresponds with low AUDPC value (92.10) and low Infection Coefficient (IC) (17.54). The Pearsons' r_{calc} values indicated a highly significant correlation among all adult plant partial resistance traits. Also, it was noticed a highly significant correlation between AUDPC values and grain yield (r = -0.9194***)

It could be concluded that the local studies on the leaf rust disease including the determination of the response of commercially rye cultivars, cultivated in marginal areas under weather factors stress, are of great benefits for both breeders and farmers, offering precious information about the impact of climatic change on the interaction between cereals and pathogens, changing host-pathogen relationship.

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