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ASSESSMENT OF LEAF RUST (*P. recondita* f. sp. *secalis*) ATTACK IN MARGINAL AREAS FROM SOUTHERN ROMANIA

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Abstract

Worldwide abiotic stress factors such as excessive temperature, precipitation, drought, salinity, soil pH, greenhouse gases, ultraviolet (UVB) radiation, and air pollution pose a persistent threat to both diseases and plants affecting host-pathogen relationship depending on geographical and temporal distribution of inoculum amount and cultivars susceptibility. Leaf rust of rye, which is caused by Puccinia recondita f. sp. *secalis (Roberge ex. Desmaz) has become one of the most important limiting factors for rye production in Central and Eastern Europe. During 2020-2021 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 to very susceptible. A negative and highly significant correlation of AUDPC with grain yield (r = -0.9222***) was found during 2020-2021 cropping season.*

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

INTRODUCTION

Climate change is affecting many aspects of our world, including agriculture and horticulture (Howden et al., 2007; Cichi et al., 2008; IPCC, 2013, Velea et al., 2021; Bonciu, 2022a; Răduţoiu and Stan, 2022).

Cereals are one of the most important crops worldwide and they are vital for human consumption and animal feed (Neupane et al., 2022).

To feed the estimated 9.8 billion people on the planet by 2050, the supply of cereal food must rise by 70-100% and 2-3% (annually) (Godfray et al., 2010; Hawkesford et al., 2013; Ray et al., 2013; Tripathi et al., 2016). According to previous studies, over the next 30 years, the earth's average surface temperature will rise at a rate of about 0.2 degrees Celsius every decade (Solomon, 2007; Bernstein et al., 2008). There are several possible strategies including breeding, technical progress and improving fertilizer and pesticides efficiency to increase crops production (Bălașu et al., 2015a; Zală et al., 2023; Sălceanu et al., 2022). The conservation of genetic resources in agriculture and food security is a long-term challenge that transcends the borders of national interests (Paraschivu et al., 2017; Cichi and Cichi, 2019; Bonciu, 2020; De Souza and Bonciu, 2022a; De Souza and Bonciu, 2022b). Maintaining access to safe, disease- and pest-free and affordable agricultural products and raw materials and ensuring sustainable agricultural production are challenges that must be faced in the context of increasing demand for agricultural products (Bonciu et al., 2021; Bonciu, 2022b).

However, despite many changes in agricultural systems as technical progress, income growth, genetically progress, improved cropping technologies, globalization on food production, machinery revolution, climate change is having a significant impact on the growth and health of cereal crops and the pathogens that affect these crops are also being affected (Partal et al., 2013; Partal et al., 2014; Cristea et al.,2015a; Cristea et al., 2015b; Paraschivu et al., 2015; Cotuna et al., 2018; Partal and Paraschivu, 2020; Păunescu et al., 2021; Paraschivu et al., 2022; Păunescu et al., 2022).

One of the most significant impacts of climate change on cereals is the increase in temperature (Chakraborty and Pangga, 2004; Chakraborty, S. & Newton, 2011; Chakraborty, 2013). Higher temperatures can lead to heat stress in plants, which can result in reduced yields and increased susceptibility to diseases (Coakley et al., 1999; Paraschivu et al., 2019a; Paraschivu et al., 2019b). For example, a 1°C increase in temperature during wheat cultivation could result in a 3-10% decrease in crop yields (Yao et al., 2012). This is because pathogens, such as fungi and bacteria, thrive in warm and moist conditions, and can infect plants more easily when they are stressed.

Another impact of climate change on cereals is the change in precipitation patterns. Changes in rainfall and humidity levels can lead to changes in the distribution and severity of plant diseases. For example, increased rainfall can create conditions that are conducive to fungal diseases, such as Fusarium head blight, which affects wheat and barley crops (Cotuna et al., 2013; Paraschivu et al., 2014; Cotuna et al., 2022b). This disease can lead to reduced yields and poor-quality grain, which can have a significant impact on farmers and the food supply. Also, 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; Răduţoiu D., 2020; Răduțoiu & Băloniu, 2021; Răduţoiu, 2022; Răduţoiu & Ștefănescu, 2022). Thus, some pathogens tend to become more aggressive even in cropping systems based on crops diversification by minor cereals.

Puccinia recondita f. sp. *secalis* (Prs) is a fungal pathogen that causes leaf rust in barley and wheat, but little is known about its host range and pathogenicity on rye.

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 and as feed for livestock. Currently rye crop 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.

One of the most important diseases 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 (Brown and Hovmøller, 2002).

Developing resistant varieties of rye is an effective way to manage leaf rust. This approach is environmentally friendly and can be a cost-effective way to manage the disease (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. Therefore, screening rye cultivars for adult plant resistance or using the marker-assisted selection in rye breeding program is of great importance to find new resistance genes associated with leaf rust resistance.

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 southern Oltenia, Romania.

MATERIALS AND METHODS

With no molecular markers or differential sets available for the identification of races in *Puccinia recondita* f. sp. *secalis* (Prs), during

2020-2021 growing season a trail for screening different rye genotypes for their adult plant partial resistance to *P. recondita* f. sp. *secalis* was carried out 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 and 83 ppm), low in organic carbon (between 0.12 and 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 selected 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). All rye genotypes were phenotyped for their adult plant partial resistance using 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).

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. Uredospores have up to eight germ pores scattered in dense walls. 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 1).

Figure 1. Uredospores pulstules on rye leaves in 2021 year (original photo: Paraschivu Mirela)

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 pretagged 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

Globally, marginal lands make up about 21 percent (2.74 billion ha) of the total land (13.5 billion ha) area. However, about 1 558 million ha of these lands are used for agriculture, out of which about 224 to 300 million ha is classified as agriculturally marginal areas (Ahmadzai et al., 2022). The term "agriculturally marginal areas" (AMAs) refers to less-favourable agricultural areas (LFAAs) that are characterized by resource degradation, constrained agricultural potential, and low productivity of agricultural resources due to biophysical constraints like rocky terrain, harsh weather, poor soil quality, salinization, drought and erratic rainfall, among other factors that pose significant obstacles for intensive agriculture.

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; West et al., 2012; Chakraborty, 2013; Elad and Pertot, 2014; Fones at al., 2020; Wolfe and Ceccarelli, 2020).

The 2020-2021 cropping season was favourable to rye Leaf rust disease in the dry area in Southern Oltenia, Romania. According to Meidaner et al. (2011), *Puccinia recondita* f. sp. *secalis* (Prs) is prolific across all rye growing regions in Europe. To date only little research was done in Europe to trigger resistance breeding. Host resistance represents a sustainable and more environmentally conscious alternative to chemical control strategies (Nelson et al., 2018). Currently, there are no newly developed rye cultivars registered that are known to carry stem rust resistances. Previously, some authors reported resistant rye plants in populations from Italy, China, Sweden, Uruguay, the Czech Republic, Azerbaijan, former Yugoslavia, Lithuania, Ukraine, Bulgaria, Portugal, Finland, Great Britain and in in South African fodder rye (Solodukhina and Kobylyansky, 2001; Boshoff et al., 2019).

To predict the Leaf rust disease development, rainfalls and temperatures were taken into account.

Humidity was determined by the amount of rain of 406.00 mm, comparatively with multiannual average rainfall of 376.85 mm, while the monthly average temperature was 13.7ºC comparatively with multiannual average temperature of 12.7ºC (Figure 2).

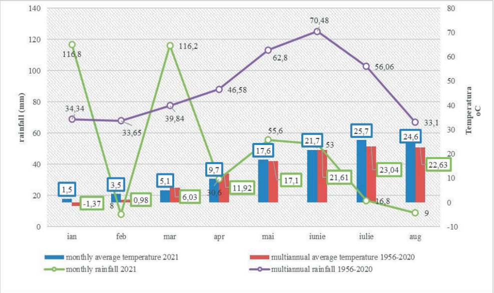

Figure 2. Climatic conditions during the study period (2021 year)

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 higher with 29.15 mm than multiannual amount for dry areas in Southern Romania, which led to symptoms exhibition on rye genotypes leaves in middle April 2021.

Starting with May 2021 the humidity decreased, while temperatures increased. The disease can result in significant yield losses over wide areas when the summers are warm and dry. 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 2 m/s. In the experimental field the wind speed ranged between 1.5-37 km/h. 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. Symptoms vary on the degree of cultivar resistance. There are cultivars that are very susceptible and have large uredinia without generating necrosis or chlorosis in the plant tissues. Different responses are used to identify resistant varieties, ranging from tiny spots to small- to medium-sized uredinia that may be surrounded by necrotic and chlorotic areas.

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 3). Previous research showed similar results on the same rye cultivars (Paraschivu et al., 2021)

Table 3. Partial resistance traits to leaf rust in adult plant of four rye genotypes

Genotype/ Type of resistance	**FRS**	**AUDPC**	**rAUDPC**	**CI**	**IR**
Binnto	23.11**	12.55***	444.22***	6.93***	0.0300*
Serafino	30.06*	23.25**	239.78**	12.02**	0.0429
Inspector	35.18	35.57	156.73	14.07	0.0542
Suceveana	43.50	55.75	100	43.50	0.0565

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 Binnto possessed high level of adult plant partial resistance based on the assessed traits, during 2021-2021 cropping season. Binnto recorded the lowest Final Rust Severity (FRS) (23.11%), which corresponds with low AUDPC value (12.55) and low Infection Coefficient (IC) (6.93). The differences for all resistance traits Binnto genotypes were highly significant comparatively with the control genotype. Rye genotypes Serafino and Inspector were race-nonspecific, moderate resistance, while the control Suceveana was susceptible, race-nonspecific, low resistance.

Among all adult plant partial resistance traits was noticed a highly significant correlation (Table 4).

Table 4. Correlation coefficients (r)* for disease parameters of leaf rust on rye genotypes at DRSPCS Dabuleni during 2020-2021 cropping season

Disease parameter	FRS	AUDPC	rAUDPC	CI	IR
FRS	1	0.994***	-0.945***	0.907***	0.943***
AUDPC		1	-0.906***	0.935***	0.912***
rAUDPC			1	-0.733**	-0.986***
CI				1	0.716**
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 rAUDPC and CI in 2020-2021 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.

Negative relation between the disease level (AUDPC) and grain yield was found. The highest significant loss percentages were found in susceptible genotypes Suceveana and moderate resistant one Serafino. The value of determination coefficient ($R^2 = 0.8505$), for all rye genotypes assessed, indicated that up to 85% 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 = -0.9222***) (Figure 3).

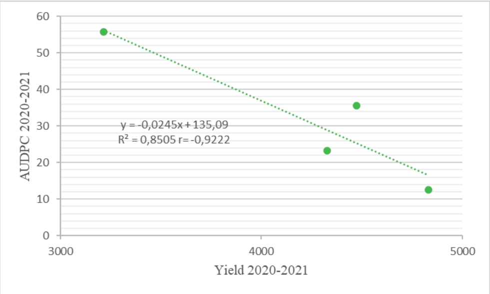

Figure 3. Relationship between Leaf rust AUDPC value and rye grain yield in 2020-2021 cropping season

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).

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 impact significantly the interaction between cereals and pathogens, changing host-pathogen relationship.

CONCLUSIONS

Leaf rust is a significant disease in rye worldwide causing significant yield losses. The present study was carried out to assess the adult plant response of four rye genotypes to the attack of *P. recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) in natural infections in dry area from Southern Romania during 2020-2021 cropping season. The response of rye genotypes to the Leaf Rust (LR) included different variation in plants reaction ranging from moderately resistant (Bintto) to moderately susceptible (Serafino, Inspector) and very susceptible (Suceveana), depending on genetic background and environmental conditions. Statistically significant differences were also observed between the control genotype (Suceaveana) and other assessed genotypes. The highest leaf rust severity was observed Suceaveana, while the lowest in Binnto. The values of Area under Disease Progress Curve (AUDPC) ranged from 12.55 (Binnto) to 55.75 (Suceveana). Binnto recorded the lowest Final rust severity (FRS) (23.11%), which corresponds with low AUDPC value (12.55) and low Infection Coefficient (IC) (6.93). 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 ($r = -0.9222^{***}$). However, under natural infection, selection on a phenotypic basis frequently results in the selection of plants that test falsely positive and does not produce adequate results. Thus, using artificial inoculation can increase selection efficiency. Therefore, investigations under both natural and artificial infections are required in order to achieve a better selection of resistant cultivars and to have a better understanding of how pathogen attacks affect grain yield.

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