GENETIC AND CHEMICAL APPROACHES TO MANAGE RYE LEAF RUST (PUCCINIA RECONDITA F.SP. SECALIS) IN NATURAL CONDITIONS FROM MARGINAL AREAS

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Abstract

*Pathogens and pests are predicted to spread to areas where they were previously irrelevant due to climate change and human-induced changes, posing new management issues for crops, especially in cropping systems based on minimal cereal crop diversification. In temperate areas of Central and Eastern Europe, rye (Secale cereale) is a minor cereal that contributes to crops diversification particularly in marginal situations where soil and climate are unfavorable for wheat production. During 2021-2022 growing season, a plant–pest-pathogen interaction profile was observed on four rye genotypes (Binnto, Inspector, Serafino, Suceveana) and also was observed the effect of different chemical and biological pesticide formulations on rye leaf rust 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. Among all evaluated rye genotypes the greatest resistance was noticed in Serafino that recorded the lowest AUDPC value (51,76), while the most susceptible was Suceveana genotype with AUDPC = 279,55. The best protection against leaf rust was provided by Poliversum (the 1st assessment – attack degree = 3,23%; the 2nd assessment – attack degree = 7,56%). Negative and significant correlation of leaf rust attack degrees with grain yield (r =-0,9393***) were found during 2021-2022 cropping season.*

Key words: *leaf rust, rye, genotype, control, genetic resistance*

INTRODUCTION

Globally, there has been a lot of interest in the potential of marginal lands to increase food security by introducing crops that cope with stress constrainers, promote the production of bioenergy, or provide ecosystem services. The term of marginal land shows up in the early 19th century in the Theory of Rent from Ricardo, D. (Ricardo, 1817), developed forward by Peterson and Galbraith in1932. The definitions of marginal land and its application domains differ across regions, countries, and organizations due to their different objectives (Baldock et al., 1996; James, 2010; Esch et al., 2018; Wells et al., 2018). Marginality is increasingly determined by factors that affect yield, such as soil sodicity, salinity, water management, and physical qualities, but can include also climatic factors, which can prevent soils from being used for traditional agricultural operations (Schubert et al., 2008; Confalonieri et al., 2014; Jones et al., 2014). The current estimate of marginal lands accounts for 36% of cultivated lands (1.3 × 109 ha), which may provide food for 1/3 of the global population (Wood et al., 2000; Kang et al., 2013). Marginal lands are associated with severe land degradation, low crop productivity, and high environmental risk, and high impact of biotic and abiotic constrainers exacerbated by climate changes (Barbier, 1989; EEA, 2017; Bonciu, 2019; Matei et al., 2020; Partal and Paraschivu, 2020; Lal, 2021; Malhi et al., 2021; Paraschivu et al., 2021).

Despite the negative effect of climate changes, agricultural production increased significantly over the past few decades as a result of numerous changes in agricultural systems brought about by the interaction of numerous factors, including globalization in food production, genetic advancement, biotechnologies, improved cropping technologies, better pest, disease and weed management and agricultural digitalization (Butnariu et al., 2006; Partal et al., 2013; Sărăţeanu et al., 2013; Partal et al., 2014; Sărățeanu et al., 2016; Bonciu, 2018; Cichi and Cichi, 2018; Cichi and Cichi, 2019a; Cichi and Cichi, 2019b; Sărățeanu et al., 2019; Bonciu, 2020a; Bonciu, 2020b; Sărățeanu et al., 2020; Bonciu et al., 2021; Bonciu et al., 2022; De Souza and Bonciu, 2022a; De Souza and Bonciu, 2022b).

Worldwide, one of the negative effects of climate change is that some pathogens and pests tend to become more aggressive even in cropping systems based on crops diversification by minor cereals (Cotuna et al., 2013; Paraschivu et al., 2015; Paraschivu et al., 2016; Cotuna et al., 2018; Paraschivu et al, 2019; Juroszek et al., 2020). Rye (*Secale cereale*) is a minor cereal, closely related to barley and wheat, having a major role in 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 tonnes of rye grains from which 71,82% was produced in Germany and Poland (USDA, 2020).

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).

In natural conditions, yield losses can be up to 40%, but they can be as high as 80% in case of early infection (Solodukhina, 2002; Wehling et al., 2003).

Despite the fact that resistance is now regarded as the disease's most cost-efficient and successful control strategy, cereal rusts have the ability to generate, recombine, and select for resistance while also adapting to new environments. Therefore, additional fungicides used is still remaining an important part of integrated disease management. The application of fungicides led to 29% higher yields comparatively with untreated plots (Hartleb et al., 1995).

Little research is reported in controlling pathogens and pests in rye system in dry marginal areas. In this context the present paper emphasises the management of rye-pathogen interaction in dry marginal environment from Southern Oltenia, Romania, using different formulations of conventional and biological pesticides and various genotypes characterized by different genetic resistance.

MATERIALS AND METHODS

During 2021-2022 growing season, a plant–pest-pathogen interaction profile was observed on Suceveana rye genotype using different chemical and biological pesticide formulations in a randomized complete block design with three replications in dry area from Research and Development Station for Plant Culture on Sands Dăbuleni, 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).

Also, in the same location a trail for screening different rye genotypes (Serafino, Bintto, Inspector and Suceveana) for their adult plant partial resistance to P. recondita f. sp. secalis was carried out in a randomized complete block design (RCBD) with three replications. Each plot had 5 m2, 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 disease frequency (F), disease intensity (I) and disease attack degree (AD%), Area Under the Disease Progress Curve (AUDPC) and relative Area Under the Disease Progress Curve (rAUDPC).

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

These parameters were used to calculate Attack Degree (AD%) using the formula: AD% = (F% x I%)/100 (Cociu and Oprea, 1989). Attack degree 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):

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

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

For the trial with chemical and biological treatments for all assessed variants were determined Frequency (F%) and Intensity (I%) of leaf rust using previous methodology. These parameters were used to calculate attack degree (AD%) (Cociu and Oprea, 1989).

The treatment combinations are presented in Table 3.

Table 3. Treatments used in the experimental trial

Factor A fungicides	Factor B insecticides
a1-no treatment	b1-no treatment
a2-Dithane M 45 - 2kg/ha	b2-Decis Expert 100 EC- 75 ml/ha
a3-Mimox - 3 L/ha	b3-Bioinsekt - 0,5-1 L/ha
	b4-Neemex - 1-1,25 L/ha

Technological measures applied included broadcasting the fertilizers at sowing time with N80P80K80, one side nitrogen fertilization during vegetation with N70, starter irrigation with 250 m3 water/ha and supplemental irrigation with 300 m3 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).

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 areas of Europe that are less suitable for conventional agriculture are growing as a result of changing climatic conditions. Therefore, finding suitable crops with profitability for these areas is a challenge for both scientists and farmers. Sandy soils from dry area in south of Romania have poor natural resources for success of conventional crops and therefore require crops that cope with limited resources and high temperatures during cropping season. Previous findings emphasized that ones of the most suitable crops for these lands are sweet sorghum, sweet potato, rye, triticale, peanuts, cowpea, Jerusalem artichoke (Prioteasa et al.2018; Prioteasa et al. 2019; Diaconu et al., 2019; Dima et al., 2019; Matei et al, 202a; Matei et al., 202b; Dima et al., 2021a, Dima et al. 2021b, Drăghici et al., 2021; Matei et al., 2021).

In dry marginal areas, especially in vulnerable crop systems like cereals, the effects of climate change and climate variability on crops health have been associated with changes in pathogens life cycles, increased incidence, pathogenicity, genetically recombination and aggressiveness traits (Chakraborty and Pangga, 2004; Newton et al, 2011; West et al., 2012; Fones at al., 2020; Cotuna et al., 2021; Paraschivu et al., 2021).

The 2021-2021 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 258,6 mm, comparatively with multiannual average rainfall of 376,85 mm, while the monthly average temperature was 14,29 ^{o}C comparatively with multiannual average temperature of 12,7 ^{o}C (Figure 1).

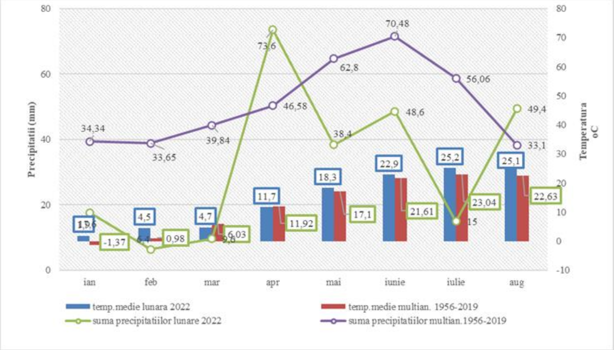

Figure 1. Climatic conditions during the study period (2021-2022 year)

During the rye cropping season the only month with higher rainfall amount than multiannual average rainfall sum (1956-2019) for the same geographic area was April (73,6 mm) which exhibited the first symptoms of leaf rust on the 4th and the 3th rye genotypes leaves.

Also, the monthly average temperature increased up to +1,5 ^{o}C comparatively with multiannual average temperature for January to August between 1956-2019.

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.

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.

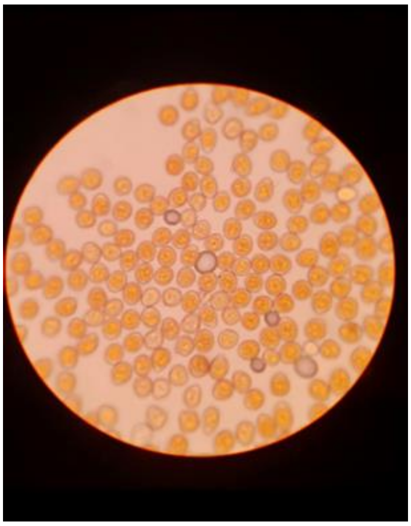

Figure 2. Uredospores of *Puccinia recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) (original photo Paraschivu Mirela)

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).

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

Thus, comparatively with Suceveana (control), only Serafino and Binnto possessed high level of adult plant partial resistance based on the assessed traits. Serafino recorded the lowest Attack Degree (AD) (4,90%), which corresponds with low AUDPC value (120,45).

The differences for all resistance traits for Serafino and Binnto genotypes were significant comparatively with the control genotype (table 4).

Rye stem rust caused by Puccinia graminis f. sp. secalis can be found in all European rye growing regions. When the summers are warm and dry, the disease can cause severe yield losses over large areas. To date only little research was done in Europe to trigger resistance breeding.

Table 4. Partial resistance traits to leaf rust in adult plant of four rye genotypes in 2021-2022

Genotype	AD	AUDPC	rAUDPC
Binnto	11,25*	120,45*	232,08*
Serafino	4,90***	51,76***	540,09***
Inspector	13,06	137,90	202,72
Suceveana	25	279,55	100 (control)

AD = Atack Degree; AUDPC = Area under disease progress curve; rAUDPC = Relative area under disease progress curve

**Significance level at P ≤ 0.01

Suceveana = control

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 value of determination coefficient (R2 = 0,9394), for all rye genotypes assessed, indicated that up to 93% 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 = -0,9591***) (Figure 3).

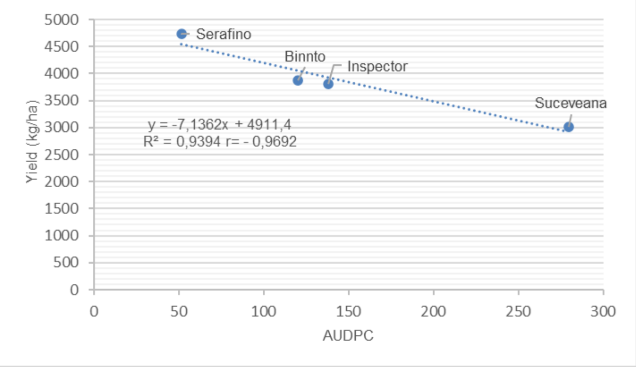

Figure 3. Relationship between Leaf rust AUDPC value and rye grain yield in 2021-2022 cropping season

During the cropping season 2021-2022 the most affected variant by the attack of leaf rust was a1b1 (control – no treatment).

The results emphasized that at the 1st determination (the beginning of booting stage) after the 1st treatment applied the incidence of leaf rust severity was low for all fungicides [Poliversum (AD = 3,23%) and Mimox (AD = 4,51%)] comparatively with the control variant (no treatment) (AD =7,53%).

It was observed that Neemex insecticide has a slightly fungicide effect when it was applied alone or mixed with fungicides (Table 5).

Table 5. The influence of the 1st treatment applied for controlling pathogens and insect's attack on rye during 2021-2022 cropping season

Fungi-cide	Insecti-cide	Attack degree the 1st det.- after the first treatment		
		Leaf rust		
		AD%	Dif. %	Signif
No treatment	Netratat	7,53	Mt	
	Decis Expert 100 EC	5,10	2,43	o
	Bioinsekt	1,02	6,51	ooo
	Neemex	4,11	3,42	ooo
Poliversum 100 g/300 l water/ha	Netratat	3,23	Mt	
	Decis Expert 100 EC	0,56	2,67	o
	Bioinsekt	0,91	2,32	o
	Neemex	1,89	1,34	o
Mimox 3l/ha	Netratat	4,51	Mt	
	Decis Expert 100 EC	1,82	2,69	oo
	Bioinsekt	1,55	2,96	oo
	Neemex	2,17	2,34	o
	LSD 5%		1,24	
	LSD 1%		2,52	
	LSD 0,1%		3,10	

It was noticed that for the second assessment (filling grain stage) the attack degree values were higher than after the first treatment due to favourable climatic conditions that favourited rust successive infections (Table 6).

Table 6. The influence of the 2nd treatment applied for controlling pathogens and insect's attack on rye during 2021-2022 cropping season

Fungi-cide	Insecti-cide	Attack degree the 1st det.- after the first treatment		
		Leaf rust		
		AD%	Dif. %	Signif
No treatment	Netratat	12,50	Mt	
	Decis Expert 100 EC	8,13	4,37	oo
	Bioinsekt	10,56	1,94	
	Neemex	7,81	4,69	oo
Poliversum 100 g/300 l water/ha	Netratat	7,56	Mt	
	Decis Expert 100 EC	3,55	4,01	oo
	Bioinsekt	4,70	2,86	o
	Neemex	5,10	2,46	o
Mimox 3l/ha	Netratat	8,12	Mt	
	Decis Expert 100 EC	4,72	3,40	o
	Bioinsekt	5,10	3,02	o
	Neemex	5,73	2,39	o
	LSD 5%		2,38	
	LSD 1%		3,72	
	LSD 0,1%		4.71	

The disease is the most infectious during heading and grain filling stages of rye development.

Winter rye varieties are generally found to be susceptible to leaf rust, so additional chemical and biological management is required.

For both treatments the best control of leaf rust was assured by Poliversum + Decis Expert 100 EC. The highest yields were obtained for the variants with fungicides mixed with insecticides (Poliversum + Decis Expert 100 EC (3317,8 kg/ha) și Poliversum + Bioinsekt (3248,9 kg/ha). Negative high correlations were observed between grain yield and leaf rust attack in 2021-2022 cropping season. These findings indicate that yield decreased due to the impact of biotic constrainers on plants which led to less healthy plant tissue available for photosynthesis. The highest significant loss percentages were found in no treated variant. The value of determination coefficient (R2 = 0,7339) indicated that up to 73% of variation in rye yield could be explained by leaf rust attack. It was noticed a highly significant correlation between leaf rust severity and grain yield (r=-0,8566***) (Figure 4).

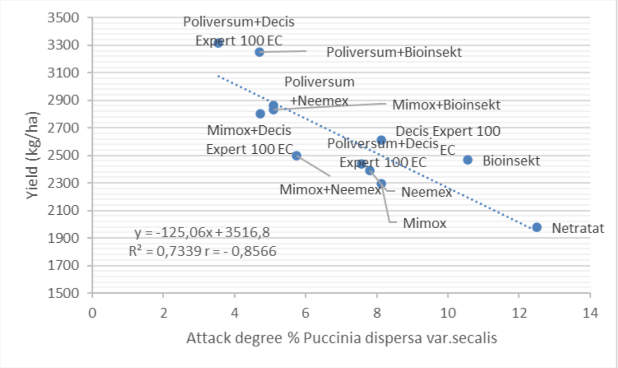

Figure 4. Relationship between Leaf rust attack degree value (%) and rye grain yield in 2021-2022 cropping season

Yield loses due to Leaf rust in rye in Europe were also reported previously by different authors (Roux and Wehling, 2010; Meidaner et al., 2012). The experiment's findings, however, demonstrate that Leaf Rust is a major disease of rye in arid marginal areas of Romania, and climate variability can cause more outbreaks.

CONCLUSIONS

The goal of the current study was to evaluate how four rye genotypes responded as mature plants to P. *recondita* f. sp. *secalis* (Prs) (Roberge ex. Desmaz) infections that occurred naturally in arid areas of Southern Romania during the 2021–2022 cropping season. The study emphasized that the increase of monthly temperature with +1 ^{o}C may lead to earlier incidence of the disease starting even with the end of April. Depending on their genetic background and climatic conditions, different rye genotypes responded to Leaf Rust infection (LR) in a variety of ways, from those that were moderately resistant (Serafino, Bintto) to those that were moderately susceptible (Inspector) and to those that were very susceptible (Suceveana). Comparatively with Suceveana genotype (control), only Serafino and Binnto possessed high level of adult plant partial resistance based on the assessed traits, during 2021-2022 cropping season. Also, it was noticed a highly significant correlation between AUDPC values and grain yield (r = -0,9692***). For both treatments the best control of leaf rust was assured by Poliversum + Decis Expert 100 EC. The highest yields were obtained for the variants with fungicides mixed with insecticides (Poliversum + Decis Expert 100 EC (3317,8 kg/ha) și Poliversum + Bioinsekt (3248,9 kg/ha). Negative high correlations were observed between grain yield and leaf rust attack in 2021-2022 cropping season (r = 0,8566).The results show that local research on the leaf rust disease, which included a determination of how commercial rye cultivars responded when are grown in marginal areas under stress from weather factors is very beneficial for both farmers and breeders, providing invaluable knowledge about the effects of climatic change on the interaction between cereals and pathogens, changing host-pathogen relationships.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Development Research Station for Plant Culture on Sands Dabuleni, Romania and was financed by the Ministry of Agriculture and Rural Development, Romania, through the ADER Project 1.4.2. (2019-2022).

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