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Pathotype diversity of *Uromyces appendiculatus* in Northeastern Bulgaria

Патотипно разнообразие на *Uromyces appendiculatus* в Североизточна България

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ABSTRACT

Bean rust caused by *Uromyces appendiculatus* (Pers.: Pers.) Unger. is one of the most devastating fungal diseases in common beans. For the plains of Bulgaria, the disease has a sporadic spread but occurs annually in The Rhodope mountains. To determine the pathotype diversity in Northeastern Bulgaria, seventeen single uredinium isolates (SUIs) were obtained from a naturally occurring pathogen population. The virulent phenotype of the isolates to the differential set referred them to 16 pathotypes of four races. The pathotypes of race 20-16 had the highest frequency of isolation (56.3%), followed by the pathotypes of race 20-18 (31.2%) and the pathotypes of races 20-0 and 20-2 (6.3%). One pathotype was referred to the Andean-specific group of the pathogen and 15 pathotypes were referred to the non-specific group of *U. appendiculatus*. All pathotypes had a completely different virulent/avirulent phenotype compared to those previously described in Bulgaria. This investigation is the first report for the distribution of pathotypes of races 20-16 and 20-18 in the country. The high pathotype diversity of *U. appendiculatus* in Bulgaria is due to genetic recombination during the sexual process which occurs in The Rhodope mountains, or to mutations in the pathogen population as a result of breeding pressure of the new varieties grown in the country.

Keywords: common bean, pathotype diversity, *Phaseolus vulgaris*, rust, *Uromyces appendiculatus*

АБСТРАКТ

Ръждата по фасул, причинявана от *Uromyces appendiculatus* (Pers.: Pers.) Unger, е едно от най-вредоносните гъбни заболявания по обикновения фасул. За равнинните части на България болестта има спорадичен характер, но се среща ежегодно в Родопите. За определяне на патотипното разнообразие на патогена в Североизточна България са изолирани 17 моносорови изолата. Вирулентният фенотип на изолатите към диференциращите сортове на ръждата по фасула отнася изолатите към 16 патотипа на четири раси. С най-голяма честота на изолиране са патотиповете на раса 20-16 (56,3%), следвани от раса 20-18 (31,2%) и раси 20-0 и 20-2 (6,3%) съответно. Един патотип е отнесен към Andean-специфичната група на патогена, а останалите 15 патотипа към неспецифичната група на *U. appendiculatus*. Всички патотипове имат напълно различен вирулентен/авирулентен фенотип в сравнение с установените до момента в България. Патотиповете на раси 20-16 и 20-18 са нови за страната. Голямото патотипно разнообразие на *U. appendiculatus* в България се дължи на генетични рекомбинации през половия процес, който протича в Родопите или на мутации в популацията на патогена под действие на селекционния натиск на новите сортове, които се отглеждат в страната.

Ключови думи: обикновен фасул, патотипно разнообразие, ръжда, *Phaseolus vulgaris*, *Uromyces appendiculatus*

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is a widespread legume crop in Bulgaria with areas over 1700 ha in 2019. (Ministry of Agriculture, Food and Forestry, 2019). Biotic and abiotic stress have a strong influence on the yield of common bean. Among the biotic factors, the most important are diseases (viral, bacterial, and fungal). Bean rust is one of the most devastating fungal diseases worldwide (Stavely et al., 1983; Liebenberg and Pretorius, 2010). In years with favorable conditions, the disease can cause up to 100% yield losses in common bean (Stavely and Pastor-Corrales, 1989). The first report of bean rust in Bulgaria is made by Kovachevski in 1930. For the plains of the country, the disease has a sporadic spread (Kiryakov and Genchev, 2001; 2004), but under favorable conditions it can cause yield losses (Kiryakov and Genchev, 2003). Bean rust occurs annually in the Rhodope mountain where *P. vulgaris* and *P. coccineus* are grown (Beleva, 2010).

The disease is caused by macrocyclic, autoaecial fungus *Uromyces appendiculatus* (Pers.: Pers.) Unger. (Harter and Zaumeyer, 1941; Stavely and Pastor-Corrales, 1989). The pathogen overwinters in plant debris as teliospores in The Rhodope mountains in Bulgaria and is distributed by the wind in other areas of the country (Beleva et al., 2010).

U. appendiculatus population shows high virulence diversity worldwide (Mmbaga et al., 1996; Jochua et al., 2008). Up to now hundreds of races and pathotypes are identified in different parts of the world (Avecedo et al., 2005; 2008; 2013; Paucar et al., 2006; Souza et al., 2007; 2013). Until 2002, seven *U. appendiculatus* races were identified in Northeastern Bulgaria (Kirkov and Genchev, 2001; 2003) based on the reaction of the differential set proposed by Stavely et al. (1983). From 2002 to 2010 nine *U. appendiculatus* races (20-0, 20-1, 20-2, 20-3, 20-19, 29-0, 29-1, 28-1, 52-3) were identified using the twelve rust differential varieties proposed by Steadman et al. (2002) (Kiryakov and Genchev, 2004; Beleva, 2010). Races 20-0, 20-2, and 20-3 were distributed in Northeastern Bulgaria and nine of them were identified in The Rhodope mountains.

Using the scale of Mmbaga et al. (1996), Beleva (2010) turned the infection type (IT) into a quantitative disease score, which allowed the grouping of isolates of each race into pathotypes having an identical virulent/avirulent phenotype to the differential varieties. The studied 110 monosorus cultures of *U. appendiculatus* were grouped into 90 pathotypes of nine races, 84 distributed in The Rhodope mountains, seven in Northeastern Bulgaria, and one pathotype was found in both areas, respectively. The huge pathotype diversity of the pathogen in Bulgaria is a result of genetic recombination during sexual reproduction which occurred in The Rhodope mountains where the pathogen overwinters in plant debris as teliospore and/or of mutation processes in the population after the pressure of grown varieties. Therefore, the pathogen population must be systematically screened and its pathotype diversity determined. The results can be used in a breeding program for common bean rust-resistant varieties which is the most economically effective and eco-friendly way to control the disease (Stavely and Pastor-Corrales, 1989; Mmbaga et al.,1996).

The present study aims to determine the pathotype diversity in the naturally occurred *U. appendiculatus* population in Northeastern Bulgaria in 2018.

MATERIALS AND METHODS

Pathogen origin

Spore mass was collected from naturally occurred *U. appendiculatus* population on varieties Skitiya and Blyan under field conditions in Dobrudzha Agricultural Institute (DAI), General Toshevo, Bulgaria. The infected leaves of each variety were shaken on filter paper. The spore mass from the two varieties was placed separately in glass bottles with silica gel, labeled and stored at -18 °C.

Single uredinium isolation

The investigation was made under greenhouse condition during 2018-2019. Varieties Skitya and Blyan were sown in plastic pods with peat mixture. Each of them was inoculated with the pathogen population collected from the same variety under field conditions.

The primary leaves of the plants were inoculated by rubbing spore suspension ($2x10^4$ spores/ml) with a paintbrush then incubated in a moist chamber for 18h (20 °C, humidity >95%). The plants were then grown at 20-25 °C (Stavely, 1983). When initial symptoms of bean rust uredinium appeared (little white spots), they were detached by cutting the leaf tissue around the spot with plastic eppendorf (1,5 ml) with wet filter paper in it (Figure 1).

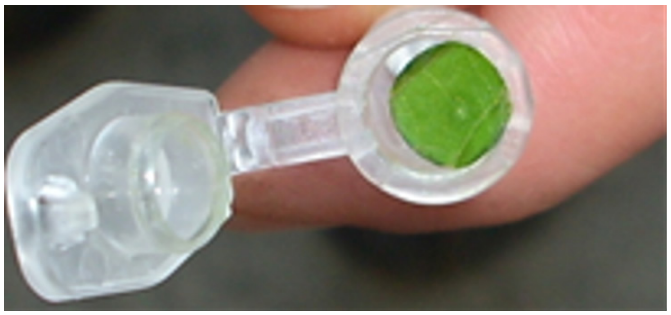

Figure 1. Single uredinium isolation with plastic eppendorf

The eppendorfs were incubated in 20-25 °C until spore mass emerged. The single uredinium isolates (SUIs) were inoculated onto susceptible variety Dobrudzhanski 7 to obtain enough spores for virulence test. The inoculation and incubation procedure is described above. The SUIs are labeled as UA.A.B.C.D, where A – the year of the collection; B – location; C – the variety of isolation; D - number of SUI.

Virulence test

The twelve bean rust differential varieties proposed by Steadman et al. (2002) were sown in plastic containers (45 x 30 x 8 cm) with peat mixture (Figure 2).

The primary leaves were inoculated with SUIs by spraying with a spore suspension. The procedure for incubation and planting were the same. The virulent phenotype of SUIs was estimated 14 days after inoculation as infection type (IT) using six-degree scale (Stavely et al., 1983) where 1 – no symptoms, Immune reaction (I); 2 – necrotic spots without sporulation (2 – spots up to 0,3 mm in diameter; 2+ - spots from 0,3 to 1,0 mm in diameter; 2++ -spots from 1,0 to 3,0 mm in diameter;

Figure 2. Common bean rust differential set

2+++ - spots over 3,0 mm in diameter), Hypersensitive reaction (HR); 3 – uredinium up to 0,3 mm in diameter, Resistant reaction (R); 4 – uredinium from 0,3 to 0,5 mm in diameter, Susceptible reaction (S); 5 – uredinium from 0,5 to 0,8 mm in diameter, Susceptible reaction; 6 – uredinium over 0,8 mm in diameter, Susceptible reaction (Table 1). IT was recorded for both leaf surfaces. If more than one IT was found on the same plant, each IT was recorded in order of its frequency. The binary system proposed by Steadman et al. (2002) was used to denote the races.

Pathotype diversity

Grouping the SUIs in races does not allow to make a thorough analysis of the virulence of the population because it is based on the susceptible reaction of the differential genotypes. This investigation grouped the isolates of each race into pathotypes based on the virulent/avirulent phenotype to the 12 bean rust differential varieties (Mmbaga et al., 1996; Avecedo et al., 2013). For this purpose, the IT of the differential varieties to every SUIs was converted to quantitative disease score (QDS) according to the scale of Mmbaga et al. (1996) (Table 2).

Data analysis

Grouping the pathotypes in the races was made by cluster analysis with program STATISTICA 7.

Table 1. Scale for estimating leaf infection type after inoculation with *U. appendiculatus*

1[1]	2	2+	2++	2+++	3	4	5	6
Immune[2]		Hypersensitive			Resistant		Susceptible	

[1] Infection type
[2] Reaction

Table 2. Infection type (IT) and quantitative disease score (QDS) in inoculation with *Uromyces appendiculatus*

IT	Description	QDS
1	No symptoms	1.1
2	Necrotic spots without sporulation	2.1
2;3	Predominant IT-2 with several IT-3	2.4
3;2	Predominant IT-3 with several IT-2	2.7
3	Uredinium up to 0,3 mm	3.1
3;4	Predominant IT-3 with several IT-4	3.4
4;3	Predominant IT-4 with several IT-3	3.7
4	Uredinium from 0,3 mm to 0,5 mm	4.1
4;5	Predominant IT-4 with several IT-5	4.4
5;4	Predominant IT-5 with several IT-4	4.7
5	Uredinium from 0,5 mm to 0,8 mm	5.1
5;6	Predominant IT-5 with several IT-6	5.4
6;5	Predominant IT-6 with several IT-5	5.7
6	Uredinium over 0,8 mm	6.1

RESULTS AND DISCUSSION

Seventeen SUIs were isolated from the investigated population of *U. appendiculatus*. The virulent phenotype of the SUIs to the differential set referred the isolates to 16 pathotypes of four races of the pathogen: 20-0; 20-2; 20-16 and 20-18 (Table 3). Races 20-0 and 20-2 were presented by one SUI and they formed individual pathotypes.

The virulence of ten SUIs classified them to race 20-16 (Table 3). The cluster analysis grouped them into nine pathotypes (Figure 3). Isolates UA.18.DAI.S.1 and UA.18.DAI.S.6 had identical virulent/avirulent phenotype and they were referred to one pathotype. Isolate UA.18.DAI.S.7 remained far from the others that formed a class in the cluster tree. Upon careful examination of QDS and IT, respectively, it was found that the main difference between the virulent phenotype of the isolates was the reaction of variety CNC, with IT = 4,3 at UA.18.DAI.S.7 and IT = 5,6; 6,5; 5,4 for the rest.

The virulence phenotype of five SUIs referred them to race 20-18 (Table 3). Cluster analysis fixed them in individual pathotypes (Figure 4). Isolate UA.18.DAI.B.3 remained far from others in the cluster tree. This is due to the reaction of differential varieties Aurora and CNC, which show IT=5,6 for this isolate and IT=4,5; 5,4 for the rest.

Ten SUIs were isolated from variety Blyan (Table 3). Their virulent phenotype referred them to ten pathotypes of races: 20-18 (four pathotypes), 20-16 (five pathotypes), and 20-0 (one pathotype). From variety Skitiya were isolated seven SUIs. The virulent/avirulent phenotype of differential varieties referred them to 6 pathotypes: four of race 20-16, one of race 20-18, and one of race 20-2 (Table 3).

The pathotypes of race 20-16 (56.3%) have the highest isolation rate, followed by pathotypes of race 20-18 (31.2%) and pathotypes of 20-0 and 20-2 (6.3%), respectively.

Many authors classified the *U. appendiculatus* isolates in two major groups: Andean-specific which overcame rust resistance genes of Andean origin and non-specific

Table 3. Reaction of the bean rust differential set to 17 SUIs of *U. appendiculatus*

Differential variety	Binary value	UA.18.DAI.B.1	UA.18.DAI.B.2	UA.18.DAI.B.3	UA.18.DAI.B.4	UA.18.DAI.B.5	UA.18.DAI.B.6	UA.18.DAI.B.7	UA.18.DAI.B.8	UA.18.DAI.B.9	UA.18.DAI.B.10	UA.18.DAI.S.1	UA.18.DAI.S.2	UA.18.DAI.S.3	UA.18.DAI.S.4	UA.18.DAI.S.5	UA.18.DAI.S.6	UA.18.DAI.S.7
Early Gallatin	1	HR[1]	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR
Redland Pioneer	2	I	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR
Montcalm	4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PC 50	8	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR
GGW	16	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PI 260418	32	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
GN 1140	1	HR	R	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR
Aurora	2	S	HR	S	HR	HR	HR	HR	HR	S	S	HR	HR	S	S	HR	HR	HR
Maxico 309	4	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Mexico 235	8	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
CNC	16	S	S	S	I	S	S	S	S	S	S	S	S	R	S	S	S	S
PI 181996	32	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
Race		20-18	20-16	20-18	20-0	20-16	20-16	20-16	20-16	20-18	20-18	20-16	20-16	20-2	20-18	20-16	20-16	20-16

[1]I-Immune; HR-Hypersensitive; R-Resistant; S-Susceptible

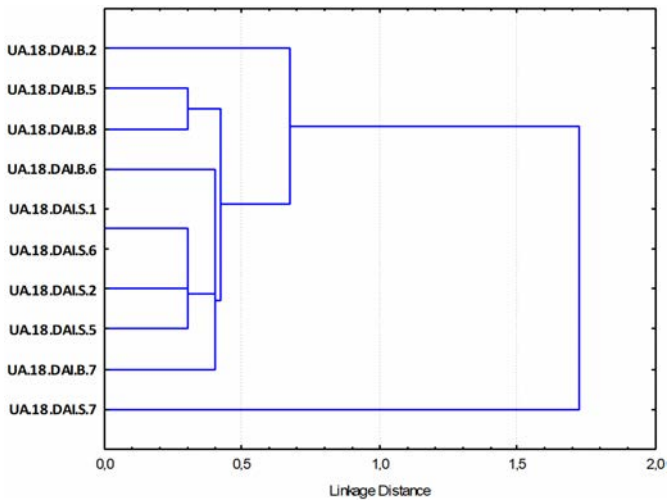

Figure 3. The Euclidean distance of ten SUIs of *U. appendiculatus* referred to race 20-16

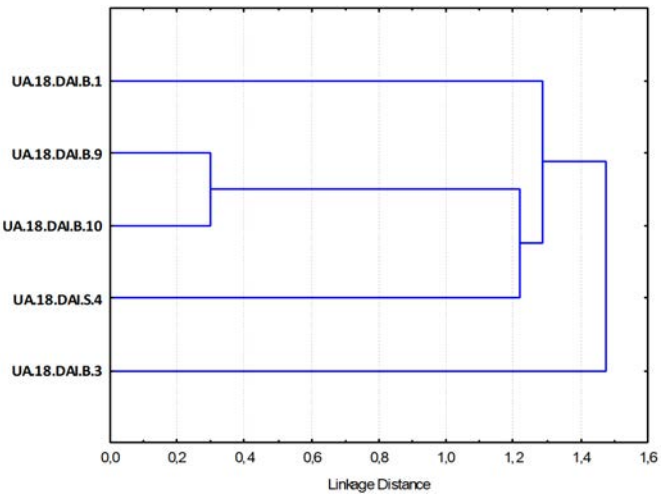

Figure 4. The Euclidean distance of five SUIs of *U. appendiculatus* referred to race 20-18

which are virulent on rust resistance genes from both (A-MA) groups and this classification was a result of the co-evolution between *P. vulgaris* and *U. appendiculatus* (Araya et al, 2004; Pastor-Corrales and Aime, 2004). The results of the present study showed that fifteen pathotypes (93,7%) overcame the rust resistance genes in the differential varieties from Andean (A) and Middle American (MA) origin and one pathotype (6,3%) overcame the resistance genes in Andean differential varieties. This gives us reason to classify 6,3% of the pathotypes (race 20-0) to A-specific group of *U. appendiculatus* and 93,7% of the pathotypes to non-specific (A-MA) group of the pathogen.

For the last twenty years, the *U. appendiculatus* population was regularly monitored in Bulgaria. The knowledge of the race/pathotype structure of the pathogen population by regions and years is of paramount importance for determining the variety structure of common bean for a specific region and provides guidelines for a breeding program for rust resistance in common bean. Races 20-0 and 20-2 were previously reported in Northeastern Bulgaria and The Rhodope mountains (Kiryakov and Genchev, 2004; Beleva, 2010). Comparative study between the pathotypes of the two races identified in this investigation with those identified by Beleva (2010) showed that they had completely different virulent/avirulent phenotypes and they are new pathotypes for Bulgaria. Moreover, this investigation is the first report for the distribution of pathotypes of races 20-16 and 20-18 in the country.

Numerous studies of *U. appendiculatus* have been conducted worldwide over the past nine years, but most have studied the genotype diversity of the pathogen using DNA markers (Odogwu et al., 2017). That is why it is impossible to compare the pathotypes identified in the present investigation with those identified in other countries. During this period no investigations were made about pathotype diversity of the pathogen in Bulgaria. At the same time, the common bean breeding program in the country was aimed at developing highly productive common bean varieties with IIa type of habitus suitable for mechanized harvesting. As a result, varieties Skitiya and Blyan were developed. They quickly replaced some of the old varieties Dobrudzhanski 7, Dobrudzhanski ran, Elixir, etc. The results from this study showed that Blyan is susceptible to four pathotypes of race 20-18, five pathotypes of race 20-16, and the pathotype of race 20-0 (Table 3). Variety Skitiya has susceptible phenotype to four pathotypes of race 20-16 and one pathotype of races 20-18 and 20-2, respectively (Table 3). Additional studies will show the response of other varieties and newly developed lines to the identified pathotypes and the reaction of Skitya and Blyan to other pathotypes of *U. appendiculatus*.

Virulence diversity of *U. appendiculatus* is a result of the action of two main factors. The first one is the life cycle of the pathogen (Avecedo, 2007). *U. appendiculatus* can survive as teliospores or uredospores in plant debris during the winter season (Stavely and Pastor-Corrales, 1989). In Bulgaria, the pathogen develops the full life cycle with five spore types in The Rhodope mountains, overwinter in plant debris as teliospore, and spread as uredospores by the wind in other areas of the country (Beleva et al., 2010). Having a full life cycle means a sexual process and genetic recombination in the pathogenic population, which leads to the emergence of new highly virulent races/pathotypes. This is why virulence diversity of *U. appendiculatus* was higher in The Rhodope mountains than in lowland areas of Bulgaria during 2007-2010 (Beleva, 2010). The second factor responsible for the appearance of new races/pathotypes is the genotype of the host (Avecedo, 2007). Resistant genotypes always suppress the pathogen population, mutation process proceeds and new highly virulent forms occur. Therefore, the existence of the new pathotypes of the pathogen in Northeastern Bulgaria is probably due to the pressure of new genotypes to the pathogen population, or the new pathotypes have come by the wind from The Rhodope mountains. It is also possible that the identified pathotypes have existed for a long time, but in a low degree of prevalence and have not been identified, but the change in the varietal structure has led to their strong multiplication and isolation, respectively.

CONCLUSIONS

The results from this investigation determine the distribution of 16 pathotypes of four races: 20-0; 20-2; 20-16 and 20-18 of *Uromyces appendiculatus* in Northeastern Bulgaria. The pathotypes of races 20-16 and 20-18 have the highest frequency of isolation.

The pathotype of race 20-0 is classified to the Andean-specific group of *U. appendiculatus*, and the other pathotypes to Andean-Middle American group of the pathogen.

This is the first report for the distribution of pathotypes of races 20-16 and 20-18 in Bulgaria.

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