

RESISTANCE IN COMMON BEAN (*PHASEOLUS VULGARIS* L.) TO *UROMYCES APPENDICULATUS* IN BULGARIA

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Abstract

Common bean rust, caused by *Uromyces appendiculatus*, is one of the most devastating diseases in common bean. Breeding for resistant cultivars is the most effective and eco-friendly method for disease control. Although there is no targeted breeding program in Bulgaria to create resistant cultivars to the pathogen, periodic screening of the virulent diversity of *U. appendiculatus* and sources of resistance to the identified races/pathotypes is carried out. During the last twenty years, four investigations were made to estimate virulence diversity in pathogen population. Eleven *U. appendiculatus* races (20-0, 20-1, 20-2, 20-3, 20-19, 29-0, 29-1, 28-1, 52-3, 20-16 and 20-18) are identified, distributed in Rhodope mountains and North Bulgaria. Chronologically each of the studies was accompanied or followed by a screening for sources of resistance to the pathogen under field conditions. Each study found different numbers of resistant accessions depending on the virulent potential of the population used. In three of the studies, cultivars that showed resistant phenotype under field conditions were screened against a set of races, distributed in the country in the same time, under greenhouse. Bulgarian cultivar 'Beslet' showed resistant reaction under field and greenhouse in all investigation. The resistance of the cultivar to races 20-1 and 20-19 is governed by a single dominant gene. This cultivar can be successfully used in a breeding program for race-specific resistance. Fifty accessions with race-nonspecific resistance, expressed by low disease intensity and resistant/susceptible infection type, are identified in two investigations under field conditions.

Key words: common bean, *Phaseolus vulgaris* L., rust, *Uromyces appendiculatus*

Common bean rust is the most devastating bean disease for many regions of the world (Stavely et al., 1983). The disease occurs primarily in North and South America, East and West Africa and Australia (Steadman et al, 2002). When it occurs early in the growing season and conditions are favorable for disease development, it can cause losses in bean yields from 25 to 100% (Stavely et al., 1983; Stavely and Pastor-Corrales, 1989).

The disease was described for the first time in Bulgaria at the beginning of the

20th century (Kovachevski, 1930). For the plain regions of the country, the disease has a sporadic character and appears late in the growing season (Kiryakov & Genchev, 2001; 2004), but in years with favorable climatic conditions, it develops epiphytotically and leads to significant losses (Kiryakov & Genchev, 2003). The disease occurs annually in the Rhodope mountains, where landraces of *Phaseolus vulgaris* L. and *Phaseolus coccineus* L. are grown without crop rotation, on small areas, in mixed populations (Genchev & Kiryakov, 2005).

Common bean rust is caused by the macrocyclic, autoaecial, phytopathogenic fungus *Uromyces appendiculatus* (Pers.:Pers.) Unger. [Syn. *Uromyces phaseoli* (Pers.) G. Wint] (Harter & Zaumeyer, 1941; Stavely & Pastor-Corrales, 1989). In Bulgaria the pathogen overwinters in plant debris as teliospores in The Rhodope mountains and is distributed by the wind in other areas of the country (Beleva et al., 2010).

Hosts of *U. appendiculatus* are species of the genus *Phaseolus* - *P. coccineus* L., *P. acutifolius* L., *P. lunatus* L., species of the genus *Vigna*. (Stavely & Pastor-Corrales, 1989).

Although, some studies show that resistance to *U. appendiculatus* is governed by one recessive gene (Zaiter et al., 1989), two genes with epistatic action (Finke et al., 1986), two complementary dominant genes (Gonzales-Garcia & Grafton, 1996), two independent genes (Grafton et al., 1985), closely linked genes or a gene block (Park et al., 1999a), in most investigations the resistance of *P. vulgaris* to *U. appendiculatus* is monogenic, following the "gene for gene" theory (Stavely, 2000) and is controlled by one dominant race-specific gene (Stavely & Pastor-Corrales, 1989; Pastor-Corrales, 2005; Beleva et al., 2009). Currently, 15 race-specific genes marked *Ur-1* to *Ur-14* are included in the gene list of *P. vulgaris* (Bean Improvement Cooperative, 2022). This enables the grouping of isolates from the pathogen population into physiological races based on their virulence to a set of differential cultivars.

Studies on the virulence diversity in the population of *U. appendiculatus* can be tentatively divided into three stages. The first stage covers the period from 1935 when Harter et al. reported the presence of physiological races in the pathogen population by 1983. During this period, over 150 races of *U. appendiculatus* were identified (Stavely, 1984), but based on the

reaction of different sets of differential cultivars and scales for estimating disease intensity and infection type. The second stage covered the period 1983-2002, when more than 100 races and 350 pathotypes of *U. appendiculatus* were identified in different regions of the world (Stavely et al., 1989) based on the reaction of a uniform set of 19 bean cultivars and scales to estimation infection type and disease intensity (Stavely et al., 1983). During this stage, the first announcement of the distribution of races BG-1 (4200000) and BG-2 (5010000) of *U. appendiculatus* in Bulgaria was made (Kiryakov & Genchev, 2001). Two years later Kiryakov & Genchev (2003) identified races 4010000, 4210000, 5010020, 5210000, 5210020, all of them distributed in Northeastern Bulgaria.

The third period began in 2002 and continues to this day. The identification of races of *U. appendiculatus* is based on the reaction of 12 differential bean cultivars, divided into two genetic groups depending on the genetic group of origin: Andean and Middle American. To date, more than 100 races and pathotypes of *U. appendiculatus* have been identified in different parts of the world (Avecedo et al., 2008; Paucar et al., 2006). In Bulgaria during this period 106 pathotypes, referred to eleven *U. appendiculatus* races (20-0, 20-1, 20-2, 20-3, 20-19, 29-0, 29-1, 28-1, 52-3, 20-16 and 20-18), distributed in Rhodope mountains and North Bulgaria, were identified (Kiryakov & Genchev, 2004; Beleva, 2010; Koleva & Kiryakov, 2020).

The use of resistant cultivars is considered the most effective and eco-friendly method bean rust control (Stavely & Pastor-Corrales, 1989). Monogenic inheritance of resistance is a prerequisite for breeding a large number of bean cultivars with race-specific resistance to *U. appendiculatus* in different parts of the world. The short period of efficacy is a major disadvantage

of the cultivars with vertical resistance, due to the recombinations and mutations in the *U. appendiculatus* population. The breeding strategy for increasing and prolonging the effectiveness of already achieved resistance is the use of genes that are effective in relation to the population of the pathogen spread in a given area and a gene pyramiding of race-specific genes in one genotype. Another disadvantage of race-specific resistance is the breeding pressure on the pathogen population, resulting in the appearance of new, more virulent races that overcome the resistance (Stavelly & Pastor-Corrales, 1989). An alternative to this method in bean rust control is breeding of varieties with partial (race-non-specific) resistance. In Bulgaria, until now, there is no targeted breeding program for developing common bean cultivars resistant to *U. appendiculatus*. However, over the past twenty years, periodic investigations have been made on the virulence diversity in the pathogen population in the country, monitoring the reaction of established cultivars, and searching for sources of resistance to the identified races/pathotypes.

Screening tests are mainly carried out under field conditions on a natural or artificially created infectious background (Sillero et al., 2006). To estimate the virulence potential in experimental populations of *U. appendiculatus*, genotypes included in the differential set, with identified resistance genes, are mainly used. The final estimation of the studied accessions is based on the infection type (IT) and disease intensity (DI) and is determined according to the scale of Schoonhoven and Pastor-Corrales (1987). Accessions showing resistant reaction under field conditions were screened under greenhouse conditions against a set of races/pathotypes (Steadman et al., 2002). The first study on the resistance of Bulgarian and foreign bean accessions to

U. appendiculatus in Bulgaria is made in 1993 by Kiryakov & Genchev (2001). The authors monitored the reaction of 47 bean accessions under field conditions to a mixture of six pure cultures referred to races BG-1 (4200000) and BG-2 (5010000) of *U. appendiculatus*. The pathogen population overcame race-specific resistance genes in differential cultivars US# 3 (*Ur-8*), Kentucky Wonder 780 (KW 780) (*Ur-4*), Golden Gate Wax (GGW) (*Ur-6*), as well as the unidentified gene in cultivar Pinto 650, included in the 1983 differential set (Table 1). Eight accessions showed an immune reaction to the pathogen, and 20 accessions showed resistance reaction, of which 11 were Bulgarian cultivars and lines. Cultivars Abritus and Prelom are currently grown in many regions of the country and show resistant reaction to the pathogen population (Table 1).

In 2004, Kiryakov and Genchev studied the resistance of 130 bean accessions to a mixture of pure cultures of races 4010000, 4210000, 5010020, 5210000, 5210020 of *U. appendiculatus*, distributed in Northern Bulgaria and identified in 2003. The authors used 12 bean rust differentials, included in the differential set from 2002, to estimate the virulence potential of the studied population against the current differential set. The results showed that the *U. appendiculatus* population overcomes race-specific resistance genes in the differential cultivars GGW (*Ur-6*), Great Northern 1140 (GN 1140) (*Ur-7*), Aurora (*Ur-3*), as well as the unidentified gene in the cultivar Montcalm. Eighteen accessions, of which two Bulgarian lines Biser and Pokrovnik, showed immune reaction to the pathogen under field conditions, and 36 accessions showed a resistant phenotype. Under greenhouse conditions, the immune cultivars under field conditions retain their immune reaction to

the newly identified in the same study races 20-0, 20-2 and 20-3.

In 2007-2008 Beleva (2010) studied 343 *P. vulgaris* accessions under field conditions. Virulence analysis of *U. appendiculatus* population spread in the experimental plot

in 2007 showed in that it overcomes race-specific genes *Ur-6*, *Ur-7*, *Ur-3*, *Ur-CNC*, *Ur-8*, *Ur-4* (KW 780), *Ur-6+* present in the differential cultivars, as well as the unidentified genes in cultivars Montcalm, Pinto 650, KW 814 and KW 765 (Table 1).

Table 1. Virulent potential of *U. appendiculatus* population used for inoculum and reaction of some Bulgarian cultivars under field conditions during 1993-2019.

Differential cultivar	<i>Ur-gene</i>	Year				
		1993	2004	2007	2008	2019
US# 3	8	S*	-	S	S	-
Canadian Small White 643 (CSW 643)	-	R	-	R	R	-
Pinto 650	-	S	-	S	S	-
Kentucky Wonder 765 (KW 765)	-	R	-	S	S	-
Kentucky Wonder 780 (KW 780)	4	S	-	S	R	-
Kentucky Wonder 814 (KW 814)	-	R	-	S	R	-
Ecuador 299	3+	R	-	R	R	-
Mexico 235	3+	R	-	R	R	-
Brown Beauty	4	R	-	R	R	-
Olathe	6+	R	-	S	S	-
AxS 37	-	R	-	R	R	-
NEP-2	3+	R	-	R	R	-
51051	3+	R	-	R	R	-
Early Gallatin	4	R	R	R	R	S
Redland Pioneer	13	R	R	R	R	R
Montcalm	-	-	S	S	S	S
Pompadour Checa 50 (PC 50)	9;12	-	R	R	R	R
GGW	6	R	S	S	S	S
PI 260418	-	-	R	R	R	R
Great Northern 1140 (GN 1140)	7	-	S	S	S	R
Aurora	3	R	S	S	R	R
Mexico 309	5	R	R	R	R	R
Mexico 235	3+	-	R	R	R	R
CNC	CNC	R	R	S	R	S
PI 181996	11	-	R	R	R	R
Abritus	-	R**	-	I***	I	R
Dunav 1	-	R	-	I	I	I
Beslet	-	-	-	I	I	I
Biser	-	R	R	I	I	-
Pokrovnik	-	R	R	I	I	-
IIRR 1426	-	R	-	R	R	-
IIRR 7585	-	R	-	I	I	-
Prelom	-	R	-	I	VS	R
Tyrnovo 13	-	R	-	VS	S	I
Trudovets	-	R	-	R	S	-
Miziya	-	-	-	R	I	VS

* The estimation of the differential cultivars is made by using infection type IT, where IT=1,2,3 is considered as resistant reaction and IT=4,5,6 is susceptible reaction (1 – no symptoms; 2 – necrotic spots without sporulation; 3 - uredinium up to 0,3mm in diameter; 5 - uredinium from 0,5 to 0,8 mm in diameter; 6 – uredinium over 0,8 mm in diameter)

** The estimation of the cultivars is made according to the scale of Schoonhoven and Pastor-Corrales by using IT and disease intensity (DI) in %.

***I – immune reaction; R – resistant reaction, S – susceptible reaction, VS – very susceptible reaction

The *U. appendiculatus* population used in 2008 was virulent to genes *Ur-6*, *Ur-7*, *Ur-8*, *Ur-4* (KW 780), *Ur-6+*, as well as the unidentified genes in cultivars Montcalm, Pinto 650 and KW 765. Ninety four (27.41%) of *P. vulgaris* accessions showed immune and 63 (18.37%) - resistant reaction to the pathogen population in 2007. In 2008, 79 accessions (23.03%) were immune and 41 (11.95%) were resistant. Sixty eight of the investigated accessions were immune to the pathogen in both years of investigations (among them cultivars Abritus, Beslet, Biser, Pokrovnik, Dunav 1, IIRR 7585). The author investigate the reaction of 53 *P. vulgaris* under greenhouse conditions to six pathotypes of races 20-0, 20-3, 29-0, 29-1, 52-3 and 20-19 identified in the same study. The results show that six accessions (AB 136, Black Turtle I, NAB 69, A769, NAB 19, Black Turtle II) are immune to the pathotypes used, and eleven accessions showed immune, hypersensitive or resistant reaction.

Koleva & Kiryakov (2021) studied the reaction of 43 bean cultivars and lines to *U. appendiculatus* under field conditions in 2019. The pathogen population showed virulent potential overcoming race-specific genes *Ur-4*, *Ur-6*, *Ur-CNC* as well as the unidentified resistance gene in cultivar Montcalm (Table 1). Twelve accessions had immune reaction (among them cultivars Turnovo 13, Beslet, Dunav 1), eight accessions had resistant reaction (among them cultivar Abritus), two accessions had middle resistant reaction. Under greenhouse conditions, the authors investigate the reaction of 12 bean cultivars developed in Dobrudzha

Agricultural Institute to eight pathotypes of races 20-2, 20-16 and 20-18, the last two of which were newly identified in the country in 2018. (Koleva & Kiryakov, 2020). Cultivars Pukliv 2, Abritus, Beslet, Trakiya, and Prelom had immune, hypersensitive, or resistant reaction to the used pathotypes of *U. appendiculatus*.

A comparative analysis between different investigations on resistance of common bean accessions to mass populations of *U. appendiculatus* under field conditions can hardly be done. The reasons for this are the different climatic conditions during the total five years of investigations (1993; 2004; 2007; 2008; 2019), the different virulent potential of the *U. appendiculatus* populations used in relation to the differential cultivars, as well as the sets of differential cultivars themselves, and also the studied accessions in most cases are also different. The population of *U. appendiculatus* used in 2007 has the highest virulent potential, and the population used in 2019 has the lowest (Table 1). It is important to note that both the differential cultivars and the test accessions in all studies often showed a different reaction in different estimations within the same year. This can be explained by climatic conditions during the growing season of common bean, as well as by the uneven distribution of individual races or pathotypes in the population of the pathogen.

Despite the already mentioned differences, Bulgarian cultivars Abritus, Dunav 1, Beslet, Biser, Pokrovnik, IIRR 1426, IIRR 7585 showed resistant phenotype during four or three of the years of investigations (Table 1). The Prelom variety showed

resistant reaction in 1993 and 2019, immune reaction in 2007 and a very susceptible reaction in 2008. Similar results were observed with cultivars Tarnovo 13, Trudovets, Mizia (Table 1) and the explanation of this again lies in the virulence potential of the used pathogen populations and climatic conditions during the season.

Therefore, results obtained under field conditions are confirmed or rejected under greenhouse conditions using a set of races/pathotypes. The results obtained in this way provide information about the interaction between race-specific gene in the cultivar and virulent gene in the pathogen, which is demonstrated only by IT (Koleva and Kiryakov, 2021).

The Pokrovnik line shows immune reaction to races 20-0, 20-2 and 20-3 (Kiryakov and Genchev, 2004), immune reaction to pathotype of race 20-19, hypersensitive to 20-3 and 52-3, and sensitive reaction to pathotypes of races 29-0, 29-1 and 20-0 (Beleva, 2010) (Table 2). The cultivar Abritus showed an immune phenotype to the three race 20-16 pathotypes used by

Koleva and Kiryakov (2021), as well as to two of the pathotypes of race 20-18. To the other two pathotypes of the same race, the cultivar shows resistant reaction, as well as to the pathotypes of races 29-0, 20-19 and 20-2 used by Beleva (2010). The cultivar shows a sensitive reaction to the pathotypes of races 29-1, 20-3, 52-3 and 20-0. Trakia cultivar is immune to the pathotypes of races 20-19, 20-3 and 20-18, sensitive to 29-0 and 29-1 and hypersensitive to all others pathotypes used by Beleva (2010) and Koleva and Kiryakov (2021). Compared by the rest, races 29-0 and 29-1 are the only ones that overcome race-specific genes *Ur-4* (Early Gallatin) and *Ur-9*, *Ur-12* (Pompadour Checa 50). Pastor-Corrales and Stavely (30) proposed a method for identifying race-specific genes using a set of races. Following this method is possible to suppose that cultivar Trakiya has a combination of race-specific genes *Ur-4* and *Ur-9* (*Ur-12* govern adult plant resistance) but additional investigations have to be made to confirm or reject this supposal (Koleva and Kiryakov, 2021).

Table 2. Reaction of five Bulgarian cultivars to 17 pathotypes of 9 races of *U. appendiculatus*

Cultivar	Pathotypes of races:																
	29-0*	20-19	29-1	20-3	52-3	20-0	20-2**	20-18	20-18	20-18	20-18	20-16	20-16	20-16	20-0***	20-2	20-3
Abritus	R***	R	S	S	S	S	R	I	I	R	R	I	I	I	-	-	-
Beslet	HR	I	H	H	I	R	H	I	I	I	I	I	I	I	-	-	-
Trakiya	S	I	S	I	HR	H	H	H	I	H	H	H	H	HR	-	-	-
Pokrovnik	S	I	S	H	HR	S	-	-	-	-	-	-	-	-	I	I	I
Prelom	-	-	-	R	-	-	H	H	I	H	R	H	I	HR	-	-	-

*Beleva (2010); ** Koleva & Kiryakov (2021); ***Kiryakov & Genchev (2004); ****I – immune; HR – hypersensitive; R – resistant; S - susceptible

The cultivar Prelom shows an immune, hypersensitive or resistant reaction to the pathotypes of races 20-2, 20-16 and 20-18 used by Koleva and Kiryakov (2021),

recently identified and distributed in North-Eastern Bulgaria (Koleva and Kiryakov, 2020).

The results presented in Table 2 from a total of three investigations show that cultivars Abritus, Trakia, Pokrovnik and Prelom do not react with an identical reaction to different pathotypes referred to the same race of *U. appendiculatus*. Koleva and Kiryakov (2021) observed similar results in cultivars Ustrem and Pukliv 2. The authors explained the different infection type as the result of a different interaction between resistance genes in the host and virulent genes in the pathogen. They confirm the position of Parlevliet (1985) that the term 'race' is a conditional concept whose designation is highly dependable by the race-specific genes in the differential set. According to the University of Cornell (2022), the race is a subspecies group of pathogens that infect a given set of plant cultivars and pathotype is an infrasubspecific classification of a pathogen distinguished from others of the species by its pathogenicity on a specific host or race is a qualitative concept and the pathotype is a quantitative concept.

The cultivar Beslet showed an immune, hypersensitive or resistant reaction to all 90 pathotypes of *U. appendiculatus* referred to races 20-0, 20-1, 20-2, 20-3, 20-19, 29-0, 29-1, 28-1, 52-3 (Beleva, 2010), as well as to the pathotypes of races 20-2, 20-16 and 20-18 used by Koleva and Kiryakov (2021). Beleva et al. (2009) found that the resistance of Beslet to races 20-1 and 20-19 is controlled by a single dominant gene. The authors included the cultivar in crosses with the differential cultivars Early Gallatin, Redland Pioneer, and CNC carrying race-specific genes *Ur-4*, *Ur-13*, and *Ur-CNC*, respectively. The results of the allelism test prove that the race-specific resistance gene in Beslet is not identical to the race-specific resistance genes in the used differential cultivars.

Despite multiple evidences of the monogenetic character of the resistance of

P. vulgaris to *U. appendiculatus*, there are some research works about race-nonspecific (horizontal) resistance in different countries and in Bulgaria. The elements of race-nonspecific resistance in *P. vulgaris* – *U. appendiculatus* patho-system are long latent period, low infection efficacy, low sporulation capacity, low infection period, and small size pustules (Habu & Zadoks, 1995). Under field conditions, it is possible to estimate only infection efficacy, expressed by DI, and pustule size, expressed by IT. Beleva (2010) identified 41 *P. vulgaris* accessions with race-nonspecific resistance to *U. appendiculatus* during 2007-2008. They showed susceptible phenotype (IT=4,5,6), except cultivars VAX4 and VAX 6 with IT=3, and DI from 5 to 25%. Koleva and Kiryakov (2021) identified nine accessions with similar susceptible phenotype (IT=4,5,6), except cultivar Ustrem, and DI from 1 to 10%. The final plant reaction of these accessions according to Schoonhoven and Pastor-Corrales (1987) is Resistant/Middle resistant. One of these accessions is cultivar Abritus. The reaction of Abritus under the greenhouse condition (Table 2) demonstrate that the cultivar has a race-specific gene for resistance to *U. appendiculatus*. Similar results were observed with cultivars VAX4, VAX 6 (Beleva, 2010) and Ustrem (Koleva and Kiryakov, 2021). This confirms the view of Geffroy et al. (2000) that race-nonspecific resistance can be race/isolate-specific. The research results obtained during the last 20 years on bean rust in Bulgaria show high virulence diversity in the *U. appendiculatus* population in the country, as well as identify resistant cultivars to the widespread races/pathotypes, as well as sources of resistance that can be successfully included in breeding programs to create varieties of common bean with high productive potential and race-specific or race-nonspecific resistance to *U. appendiculatus*.

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