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Common bean lines resistant to *Uromyces appendiculatus*

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Abstract: *Common bean rust, caused by basidiomycete fungus Uromyces appendiculatus is a major concern for common bean production worldwide. It occurs sporadically in the plains of Bulgaria but for the The Rhodope Mountains it is of economic importance and appear annually. The use of resistant cultivars is the most cost-effective strategy to manage bean rust disease. The aim of the present study is to screen common bean lines to several pathotypes of U. appendiculatus to find a source of resistance. The investigation was carried out during 2023 at the Laboratory Complex of the Dobrudzha agricultural institute, General Toshevo. Twelve breeding lines of common bean were inoculated with eight pathotypes of races 20-2,20-16 and 20-18. The reaction of lines was recorded 14 to 15 days after inoculation as infection type (IT) using six-point scale. The results showed that lines can be divided into three groups. The first group includes four of the P. vulgaris lines (DG 17-38-16; DG 17-38-38; DG 17-38-77; DG 17-38-9) which were immune, hypersensitive or resistant to all used U. appendiculatus pathotypes. The second group consists of three lines (DG 17-38-27, DG 17-38-28, and DG 17-38-46) which showed sensitive phenotype to all used pathotypes. The third group includes five lines with a mixed type of reaction to the studied pathotypes. The lines from the first group can be successfully used as a donor parent in a breeading program for developing common bean cultivars resistant to U. appendiculatus.*

Keywords: common bean, rust, resistance, *Phaseolus vulgaris*, *Uromyces appendiculatus*

Introduction

The common bean (*Phaseolus vulgaris* L.) is essential for global food sustainability, as it is nutritionally and economically important in many countries around the world [1]. This crop is traditional for Bulgaria. The size of the areas sown with common beans in the country varies over the years due to various reasons. In 2023, 1 389 hectares of common bean were sown in the country, and the average yield reached 993 kg/ha [2]. The

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official variety list includes six varieties of common bean, of which five are breed of Dobrudzha Agricultural Institute (DAI), located in town of General Toshevo (Eliksir, GTB Blyan, GTB Skitiya, GTB Ustrem, GTB Vezhen), and the sixth is breed of the Institute of Agriculture and Seed Science "Obraztsov Chiflik", located in town of Rousse (Obraztov chiflik 12) [3].

A myriad of abiotic and biotic factors limit common bean production in the world and lead to significant yield losses up to 67% depending on the cultivar, crop age at the time of infection, and environmental conditions [4, 5, 6].

Common bean rust, a disease caused by the biotrophic basidiomycete fungus *Uromyces appendiculatus* is a major concern for common bean production worldwide [7, 8]. The pathogen flourishes in temperatures between 17–25 °C with high humidity (>95%) [1]. More than 90 races (virulence phenotypes) of the pathogen are reported in different parts of the world which reveal the rich virulence diversity of *U. appendiculatus* population [5, 9, 10].

Bean rust occurs sporadically in the plains of Bulgaria but for the The Rhodope Mountains it is of economic importance and appear annually [11, 12, 13]. Beleva [13] determined that the pathogen overwinters in plant debris as teliospores in The Rhodope Mountains and is distributed by the wind in other areas of the country. Several works investigated *U. appendiculatus* virulence diversity in Bulgaria and determined 106 pathotypes, referred to eleven 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 [12, 13, 14].

Genetic resistance is the most cost-effective strategy to manage bean rust disease [5, 8]. The resistance of *P. vulgaris* to *U. appendicuatus* is monogenic and in most cases is controlled by one dominant race-specific gene [15, 16, 17], labeled with *Ur*-symbol [18]. The *P. vulgaris* gene list consists of 15 race-specific genes marked Ur-1 to Ur-14 [19].

Three of the six bean varieties listed on the national list (Eliksir, GTB Blyan, GTB Skitiya) had susceptible phenotype to eight of the most recently identified pathotypes of *U. appendiculatus* in the country [20]. Cultivar GTB Ustrem had resistant reaction to four of them [20] but the reaction of GTB Vezhen and Obraztsov Chiflik 12 is still unknown. Although there is no breeding program in the country to create common bean varieties resistant to rust, over the years a number of studies have monitored the resistance of bean germplasm to the pathogen with a view to searching for sources of resistance.

The aim of the present study is to screen common bean line reaction to several pathotypes of *U. appendiculatus* to find a source of resistance.

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Materials and Methods

The investigation was carried out during 2023 at the Laboratory Complex of the Dobrudzha Acricultural Institute, located in town of General Toshevo. Twelve breeding lines of common bean were grown in plastic terrines (45 x 30 x 8 cm) in three replications with a peat-perlite mixture (3:1) at 20-25°C daytime and 16-18°C nighttime temperature in greenhouse.

Eight pathotypes of races 20-16, 20-18 and 20-2 of *U. appendiculatus*, originating from Northeastern Bulgaria was used. The virulence potential of the pathotypes in relation to the twelve bean rust differential varieties is presented in Table 1 [14]. Pathotypes are labeled as A№/D, where A – cultivar from which it was isolated and serial number of the pure culture (B – Blyan; S – Skitiya), D – race of *U. appendiculatus*. Pathotypes are stored in glass ampoules containing 1g of silica gel in a refrigerator at -18°C.

Table 1. Virulence potential towards differentiating bean rust varieties of eight pathotypes of races 20-16, 20-18 and 20-2 of *U. appendiculatus*

Differential varieties	**Binary value**	**Pathotypes**							
		B2/ 20-16	**B8/ 20-16**	**B6/ 20-16**	**B1/ 20-18**	**B3/ 20-18**	**S4/ 20-18**	**B10/ 20-18**	**S3/ 20-2**
Early Gallatin	1	HR*	HR	HR	HR	HR	HR	HR	HR
Redland Pioneer	2	HR	HR	HR	I	HR	HR	HR	HR
Montcalm	4	S	S	S	S	S	S	S	S
PC 50	8	HR	HR	HR	HR	HR	HR	HR	HR
GGW	16	S	S	S	S	S	S	S	S
PI 260418	32	I	I	I	I	I	I	I	I
GN 1140	1	R	HR	HR	HR	HR	HR	HR	HR
Aurora	2	HR	HR	HR	S	S	S	S	S
Maxico 309	4	I	I	I	I	I	I	I	I
Mexico 235	8	I	I	I	I	I	I	I	I
CNC	16	S	S	S	S	S	S	S	R
PI 181996	32	I	I	I	I	I	I	I	I

I – immune; HR – hypersensitive; R – resistant; S - susceptible

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To obtain fresh spore mass, the bean rust-susceptible cultivar "Dobrudzhanski 7" was used. The plants were grown in plastic pots (with a capacity of 1L) on a peat-perlite mixture (3:1). The primary leaves of plants were inoculated by smearing the upper and lower leaf surfaces with a paintbrush with a spore suspension (2.0 x 10^4 uredospores/ml), to which 0.1% Tween 20 was added as an adhesive. After inoculation, the plants were covered with an insulator and placed in a humid chamber (20°C, relative humidity >95%) for 18h, after which they were grown under the conditions described above until the spores completely burst [21]. The fresh spore mass was collected by shaking on aluminum foil and stored in glass ampoules with silica gel in a refrigerator at 7°C until use.

The studied accessions were inoculated in the same phenophase as Dobrudzhanski 7 plants by spraying with a nebulizer of a spore suspension with a concentration of 2.0 x 10^4 uredospores/ml, to which 0.1% Tween 20 was added as an adhesive. After inoculation, the plants were incubated and grown as previously described.

The reaction of the investigated lines was recorded 14 to 15 days after inoculation as infection type (IT). A six-point scale was used, where 1 – no symptoms (Immune reaction); 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; 2+++ - spots over 3,0 mm in diameter) (Hypersensitive reaction); 3 – uredinium up to 0,3 mm in diameter (Resistant reaction); 4 – uredinium from 0,3 to 0,5 mm in diameter (Susceptible reaction); 5 – uredinium from 0,5 to 0,8 mm in diameter (Susceptible reaction); 6 – uredinium over 0,8 mm in diameter (Susceptible reaction) [13, 14, 21]. The IT was recorded on both leaf surfaces. All observed types were recorded, considering the frequency of their occurrence, separately for the two leaf surfaces (upper side/lower side).

Results and Discussion

The reaction of the twelve lines included in the investigation to eight pathotypes of *U. appendiculatus* conditionally divides them into three groups.

The first group includes four of the *P. vulgaris* lines (DG 17-38-16; DG 17-38-38; DG 17-38-77; DG 17-38-9) which were immune, hypersensitive or resistant to all used *U. appendiculatus* pathotypes (Table 2).

The second group consists of three lines (DG 17-38-27, DG 17-38-28, and DG 17-38-46) which showed sensitive phenotype to all used pathotypes.

The third group includes five lines with a mixed type of reaction to the studied pathotypes.

Line DG 17-38-19 is sensitive to the three pathotypes of race 20-16 and the only pathotype of race 20-18. As for the remaining pathotypes of 20-18, as well as to 20-2, the line shows a resistant phenotype.

Line DG 17-38-3 is resistant to two of the pathotypes of race 20-16 and three of the pathotypes of 20-18, and to the remaining pathotypes it is sensitive.

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Line DG 17-38-31 showed sensitive reaction to three of the pathotypes of 20-18, and to all the remaining pathotypes it shows a resistant or hypersensitive reaction.

Line DG 17-38-44 is resistant to one of the pathotypes of 20-16, one of those of 20-18 and the pathotype of race 20-2. The line showed a sensitive reaction to the remaining pathotypes studied.

Line DG 17-38-51 showed an immune reaction to the pathotype of race 20-2 and some of the pathotypes of race 20-16 and was resistant to the pathotype of race 20-18. The line showed a sensitive reaction to all other pathotypes (Table 2).

Table 2. Reaction of twelve common bean lines (*P. vulgaris*) to eight pathotypes of races 20-2, 20-16 and 20-18 of *U. appendiculatus* under greenhouse conditions

№	Line	Pathotypes							
		B2/ 20-16	**B8/ 20-16**	**B6/ 20-16**	**B1/ 20-18**	**B3/ 20-18**	**C4/ 20-18**	**B10/ 20-18**	**C3/ 20-2**
1	DG 17-38-16	3,4/3,4	2+/2,2++	3/3	3,4/3,4	2+/2++	2/2	1	3,4/3,4
2	DG 17-38-38	2+,2/2+	2/2	3/3	2+,2/2+	2/2	2+/2+	3,4/3	2/2
3	DG 17-38-77	1	2,3/3	3/3	3,4/3,4	2+,2/2+	3/3	2++/2++	2/2
4	DG 17-38-9	3,4/3,4	2+,2/2+	2,3/2	3,4/3,4	3/3,4	2++,2++/2++	2+/2+,2++	1
5	DG 17-38-27	5,4/5,4	5,6/5,6	5,6/5,6	5,6/5,6	5,4/5,4	4,5/5	4,5/10	5,6/5
6	DG 17-38-28	5,6/5,6	6/6	5,6/5,6	4,5/5	5,6/5,6	5,6/5,6	5,6/5	5,6/6
7	DG 17-38-46	5,6/5,6	5,4/5,6	4,5/5	5,6/5,6	4,5/5,6	4,3/4	4,3/4	4,3/4,3
8	DG 17-38-19	4/4,5*	5,4/5,4	4,5/4,5	5,6/5,6	3,2+/3,2++	3,4/3,4	3/3	3,4/3
9	DG 17-38-3	5,4/5,4	3,4/3,4	3/3	3,4/3,4	4,5/5	3,2/3	2,3/3	4,3/3,4
10	DG 17-38-31	3,4/3,4	3,4/3,4	3/3	5,6/5,6	5,4/5,4	2,2+/2+	5,6/6	2+,2/2+
11	DG 17-38-44	5,6/5,6	4,5/5	3/3	5,4/5,4	5/5,4	3/3	5,6/5,6	1
12	DG 17-38-51	4,5/5	5/5,6	1	5,4/5,4	4,5/5	4,3/4	3,4/3,4	1

*1 – no symptoms (immune); 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; 2+++ - spots over 3,0 mm in

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diameter) (hypersensitive); 3 – uredinium up to 0,3 mm in diameter (resistant); 4 – uredinium from 0,3 to 0,5 mm in diameter (susceptible); 5 – uredinium from 0,5 to 0,8 mm in diameter (susceptible); 6 – uredinium over 0,8 mm in diameter (susceptible)

Except for line DG 17-38-31 from the third group, all lines included in the first and third groups showed different IT towards the pathotypes of the same race. Similar results were obtained by Koleva & Kiryakov [20], who studied the reaction of twelve common bean varieties to the same *U. appendiculatus* pathotypes. The authors support the position of Parlevliet [22] that the term 'race' is a conditional concept and strongly depends on race-specific genes in the used differential set.

In the *P. vulgaris – U. appendiculatus* pathosystem, the designation of the races is based on the sum of binary values of each susceptible differential cultivar in the two gene pools: Andean and Middle American. The resistant phenotype can be result of immune (IT=0), hypersensitive (IT=2) or resistant reaction (IT=3) which is result of different type of interaction between resistant genes in the host and avirulent genes in the pathogen. Some research works established the importance of many other genes in common bean – rust interaction. Ayyappan et al. [23] reported that 90 genes are differentially expressed at 0, 12 and 18 hours after inoculation, including genes involved in stress responses such as calmodulin, cytochrome P450, chitinase, DNA polymerase II, and LRR, as well as transcription factors including WRKY, bZIP, MYB, HSFB3, GRAS, NAC, and NMRA. Thibivilliers et al. [24] reported up to 90 genes mainly attributed to stress response, hormone regulation/signaling, transport, and cell wall formation [25].

According to Leitão et al. [26] hypersensitive phenotype can be early-acting and complete, or late-acting and incomplete. The incomplete allows some rust development with reduced disease severity but with rust pustules associated with some levels of necrosis (reduced infection type), any case, being race specific. The authors found the incomplete hypersensitive resistance is oligogenic inherited. Incomplete hypersensitive resistance is often found in many investigations in differential cultivars (especially Redland Pioneer) and common bean lines inoculated with Bulgarian *U. appendiculatus* pathotypes (Koleva, unpublished data). In this investigation this type of reaction was observed in line DG-17-38-9, from the first group, when inoculated with pathotype C4/20-18. Probably the resistance of this line to pathotype C4/20-18 is oligogenic inherited.

It is also important to notice that all lines included in this study derived from the cross "Beslet" x "Skitiya". Cultivar "Skitiya" is susceptible to the used pathotypes [20]. Cultivar "Beslet" have resistant phenotype (IT = 1, 2 or 3) to all pathotypes of *U. appendiculatus* found in Bulgaria [13, 21]. Beleva et al. [28] found that the resistance of cultivar "Beslet" to races 20-1 and 20-19 is controlled by a single dominant gene which is different from *Ur-4* (Early Gallatin), *Ur-13* (Redland Pioneer) и *Ur-CNC* (CNC). The resistance of the cultivar to all pathotypes of the pathogen in the country supposed that the gene is different from the race-specific

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genes *Ur-3* (Aurora), *Ur-4* (Early Gallatin), *Ur-6* (Golden Gate Wax), *Ur-7* (Great Northern 1140), *Ur-9* (Pompadour Checa 50), *Ur-CNC* (CNC), Ur-260 (PI 260418) [13]. Probably lines DG 17-38-16, DG 17-38-38, DG 17-38-77, DG 17-38-9 which have resistant phenotype to eight pathotypes of *U. appendiculatus* have inherited the same gene for resistance.

Even though through the years in Bulgaria many sources of resistance to *U. appendiculatus* were found, little is known about genetic basis of this resistance. Additional investigations have to be made to determine the genetic basis of resistance of common bean lines to *U. appendicularus* to successfully include them in a breeding program.

Conclusion

The results of the investigation of 12 breeding lines of common bean (*Phaseolus vulgaris*) against eight pathotypes of *Uromyces appendiculatus*, referred to races 20-2, 20-16 and 20-18, give us reason to draw the following conclusions:

Lines DG 17-38-16, DG 17-38-38, DG 17-38-77 and DG 17-38-9 are resistant to the included pathotypes of races 20-2, 20-16 and 20-18.

The identified lines resistant to the studied pathotypes can be successfully used as resistance donors in a breeding program aimed at developing common bean varieties resistant to *U. appendiculatus.*

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