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Sources of Resistance to Common Bacterial Blight, Halo Blight and Bacterial Wilt in *Phaseolus vulgaris* L. Elite Lines

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Abstract

Field inoculation tests were performed in 2009-2013 with 32 snap bean lines *(Phaseolus vulgaris* L.*)* from six crosses for their leaf and pod reaction towards two isolates of *Xanthomonas axanopodis* pv. *phaseoli [Xap]* – the causal agent of common bacterial blight, and two races of *Pseudomonas savastanoi* pv. *phaseolicola [Psp]* - the causal agent of halo blight. The disease response of the lines to an isolate of *Curtobacterium flaccumfaciens pv. flaccumfaciens [Cff]* – the causal agent of bacterial wilt was also studied. A differential reaction of lines to *Psp* races was observed for each of the reactions on leaves and pods. Responses of the lines to bacterial wilt differed by each of the symptoms – necrotic lesions, stunting and wilting. A positive correlation (r= 0.89) between the genotype disease reaction and the virulence of the *Xap* isolates included in the study was obtained. Among the studied genotypes, three lines of hybrid combination #952 demonstrated high_ to moderate_ resistance on both leaves and pods to *Psp* and *Xap*. They possessed green flat snap pods, determinated growth habit *Ia* and good agricultural characteristics. Three lines resulting from crosses #1074 and #1056 were highly to moderately resistant to halo blight and bacterial wilt. Above outlined genotypes were suitable sources of resistance to bean bacterioses in Bulgaria and their incorporation in the snap bean breeding program would allow selecting of cultivars with resistance to a considerable part of the pathogens' populations.

Key words: green bean, breeding lines, resistance, bacterioses, yield, quality

Introduction

Bacterial diseases are major limiting constrains in common bean (*Phaseolus vulgaris* L.) crops in Bulgaria. Yield loses due to bacterioses depend of pathogen, variety resistance and environment; they may vary up to 45 % (Singh and Schwartz, 2010). At the present, three phytopathogenic bacteria were established in the country – *Xanthomonas axonopodis* pv. *phaseoli (Xap)*, *Pseudomonas savastanoi* pv. *phaseolicola (Psp)* and *Curtobacterium flaccumfaciens* pv. *flaccumfaciens (Cff)* (Kiryakov, 1999).

Halo blight (HB) caused by phytopathogenic bacteria *Psp* is a predominant bacterial disease in the years with humid and cooler climate conditions. Pathogen's population in Bulgaria consists of strains allocated to five out of nine races identified worldwide – race 1, 2, 6, 7 and 9 (Taylor et al. 1996a; Kiryakov, 2001). Race 6 is the predominant one in the country, followed by race 1 (Kiryakov, 2001). Common bacterial blight (CBB) caused by *Xap* dominates in bean crops around the country during wormer and humid years. Pathogen's population identified includes normal and *fuscans* strains, as normal are the predominant ones (Kiryakov, I., 1999). Bacterial wilt (BW) incited by pathogenic bacteria *Cff* appears sporadically in Bulgaria and it is favored by water deficiency and heat stress conditions. Pathogen population includes mainly strains with yellow pigmentation (Kiryakov, 1999).

All of the three pathogens are seed-transmitted; therefore their effective management requires using of pathogen - free seeds, as well as crop rotation and deep tillage. However, the most effective and sustainable management strategy is breeding of resistant cultivars (Singh and Schwartz, 2010). The reaction of *Phaseolus* beans to bacterial pathogens have been reported to be both quantitatively (HB, BW) or/and qualitatively (HB, CBB, BW) inherited (Coyne et al. 1965, 1966; Aggour and Coyne, 1989; Asensio et al., 1993; Arnaud-Santana et al., 1994; Taylor et al., 1996b; Genchev and Kiryakov, 2001; Singh and Schwartz, 2010).

In an attempt to breed improved cultivars adapted to Bulgarian climatic conditions that meet the requirements of can green beans with multiple bacterial disease resistance, a hybridization program was carried out at “Maritsa” Vegetable Crops Research Institute (VCRI) in Plovdiv. Crosses were made between the well adapted, best-yielded Bulgarian old varieties, but susceptible to bacterial pathogens and breeding lines that possessed resistance to halo blight (Sofkova and Poryazov, 2004) and common bacterial blight (Sofkova and Poryazov, 2004a). A systematic search and selection was made in F_5-F_{10} generations for lines that would grow and yield well in arid and moderate- to high- temperature conditions in Bulgaria (unpublished data).

Current study aimed to: i) identify disease reaction to HB, CBB and BW of green bean breeding lines from $F_{8\text{-}11}$ advanced generation; ii) select recombinants with multiple resistance towards tested bacterial pathogens and snap pods of required yield and quality.

Material And Methods

Plant materials Thirty–two *Phaseolus* bean lines of $F_{8\text{-}11}$ advanced generation from six crosses were included in the study (Table1). One of their parents is a snap bean variety with green flat pods with excellent yield and quality characteristics, well-adapted and widely grown in Bulgaria in the past. The other parent is breeding line of snap or dry bean type with HB or/and CBB resistance/tolerance transferred in a backcrossing program from lines: HBR72, RRR46 (University of Wisconsin, USA); RH26, RH13 and GY14E (Versailles, France); XAN 159 and 161 (CIAT, Columbia), (table 1).

Field inoculation tests. Inoculation tests were performed in the pathogenic fields at Dobrudzha Agriculture Institute (DAI) in General Toshevo. Plants from each line were sown at 10 cm space within rows, 0.5 m apart and 1 m long in two replications. The lines were sown at two rows per replication in the BW test (one of them used as standard checks).

Halo blight and common bacterial blight. The multiple- needle inoculation method was used to inoculate two leaflets of the fully expanded trifoliate leaf at button (HB) and flowering (CBB) with virulent strains of *Psp* and *Xap,* respectively (Kiryakov, 1999). Two pods per plant were inoculated at filling stage by puncturing with 1 ml syringe needle. Ten plants of each combination *pathogen/line/replication* were inoculated with pathogen strains PB 9941/race 1 and PB 9921/race 6 of *P.s.*pv.*phaseolicola*, and isolates XB 96221 and XB 99132 of *X.a.*pv.*phaseoli*. Bacterial suspension with concentration of 10^8 cfu/ml diluted from 24-48 hrs –old bacterial culture grown on YDC plates was used for inoculum.

Leaf and pod reactions were recorded 14 days after inoculation (DAI) according to 1-9 degree scale of Kiryakov, 1999. Disease development was determined according to mean disease index (MDI) equation: $MDI= \sum(n \times DI)/N$, where n – number of inoculated plants, DI – disease index/score, N – total number of plants. For MDI=1.0 – immune (I) genotype; for MDI = 1.1-3.0 resistant (R) genotype, and for MDI=3.1 – 5.0 moderately resistant (MR) genotype.

Bacterial wilt. Plants were inoculated ten days after emergence in the cotyledonary node using 1 ml syringe with needle (Kiryakov et al., 2010). Bacterial suspension with a concentration of 10^8 cfu/ml diluted from two day - old bacterial culture from *Cff* -isolate CC 96201 grown on YDC plates was used for inoculum. Control plants (inoculated with sterile water) from each genotype were used as standard checks as a guide in assessing stunting reaction of the same genotypes.

Disease reaction was rated 28-30 days later on the 1 to 9- degree scale for each symptoms (wilting and stunting) where: 1- no symptoms and 9- dead plant. Plant wilting index (PWI), Plant stunting index (PSI) and Mean disease index (MDI) were calculated according to Kiryakov et al., (2010). Genotypes with MDI ≥ 3.0 were considered as resistant.

Yield and quality trails. Three year-trails were conducted on the breeding fields at Maritsa VCRI, Plovdiv to evaluate 12 plants from each genotypes according to bean breeding scale for traits evaluated by plant breeders in snap beans for processing (http://bic.css.msu.edu/_pdf/Bean_Breeding_Scales.pdf): yield of fresh pods, g plant^{-1}; yield of standard pods, %; pod distribution, cm; pod suture string (0 = stringless; 3 few strings; 5 = moderately stringy; 7 = very stringy); pod length and width, cm. Sensory evaluation was performed on a randomised sample of 0.300 kg of 3 cm sliced bean pods cooked right after harvesting. Sensory characteristics including crispness, parchment layer free and stringless, were evaluated using a five-point panel test with 0.25-step.

Sstatistical analysis. Analyses of variance by Duncan’s multiple range tests (1955) and correlation of data were conducted using SPSS.12 for Windows.

Table 1. Common bean lines used in the investigation

Reg. of crosses	Crossing combination	Number of lines	Sources of resistance*
IP 943	(IP 620-1 / v.Starozagorski cher)	2	v.Perun (Maritsa VCRI, BG); HBR72 (Wisconsin, USA)
IP 952	(IP 190a-6 / IP 468-6)	10	XAN161 (CIAT); RH26 (France)
IP 989	(IP 190a-4 / v.Starozagorski cher)	9	RH26 (France) GY14E (France)
IP 1028	(IP 460-1 / IP 213-43)	1	XAN 159 (CIAT); RRR 46 (Wisconsin, USA)
IP 1056	(IP 561-1 / v. Trakiiski)	10	RH13 (France)
IP 1074	(IP 612-1 / IP 213-43)	3	v. Fiesta (Maritsa VCRI, BG); RRR 46 (Wisconsin, USA)

*: Sources of resistance to *P.s.* pv. *phaseoli* and *X.a.* pv. *phaseoli*

Results

Resistance to Halo blight. No differences in the degree of virulence were observed between the *Psp* isolates over all lines for their leaf reaction (table 2). Race 1 of *Psp* was significantly more virulent over all lines in their pod reaction compare to race 6.

Immune and resistant reaction on leaf to race 1 of *Psp* showed five and seven of the tested lines, respectively (table 2). Line IP 952-61 had immune and line IP 952-55 – resistant reaction on the pod to the same race. Total of 15 lines showed immunity, resistance or medium resistance to race 1 of HB both on pod and leaf.

Disease reaction to race 6 of *Psp* was as follows: 12 lines with leaf resistance, two with immune leaf reaction and 12 lines with pod resistant reaction. Immune, R or MR reaction to that isolate on both leaf and pod showed 21 genotypes. Recorded results reviled that twelve out of 32 lines included in the study had combined immune to medium resistant phenotype to races 1 and 6 of halo blight both on pod and leaf.

Resistance to Common bacterial blight. Leaf reactions of the lines to the different isolates of *Xap* were significantly different, with the isolate XB99132 being more virulent (table 2). No differences in the degree of virulence were observed among the *Xap* isolates over all lines for their pod reaction.

Immune and resistant leaf reaction to isolate XB96221 of CBB showed four and six lines respectively (Table 2). Line IP 952-58 was the only one with resistant reaction of pods to that isolate, and line IP 952-56 и IP 952-67 had MR reaction. Resistant leaf reaction to isolate XB99132 of *Xap* had five lines with three of them having resistance on pods as well (IP 952-60, IP 952-61 and IP 989-118). Genotype IP 952-55 showed resistant foliage reaction and MR reaction on pods; and line IP952-58 – MR and R reaction on leaves and pods respectively. A total of eight lines had immune to MR phenotype reaction on pods and leaves to all of the studied *Xap* isolates.

Resistance to Bacterial wilt. Differences in PWI and PSI were recorded in overall numbers of lines included in the test (Table 2). No symptoms of wilting or/and stunting were observed in lines IP 952-70-3, IP 1056-253 and IP 1074-264. Twelve lines showed resistant phenotype to BW having MDI≥3.0.

Yield and quality. Data recorded for the economically yield and quality characteristics are shown on Table 3. Significant differences were observed in yield of fresh pods per plant, with highest value recorded for line IP 1074-263 (153,8 g) and lowest – in lines IP 952-60 (98.9 g) and IP 952-61 (105.6 g). Pods length and width vary from 118 to 153 mm and from 10 to 16 mm respectively. Overall distribution of pods observed was higher that 19-20 cm of the plant stem.

Breeding for disease resistance is the main strategy for sustaining and long term control of diseases in bean crops (including bacterial pathogens), with multiple resistance towards two or more isolates/races/pathogens has been preferred (Singh and Schwartz, 2010). Analysis of the data recorded reviled that six from the tested lines were with resistant pod and leaf phenotype (I-MR) to races 1 and 6 of *Psp* along with two highly virulent isolate of *Xap* (table 2). However none of them were resistant to BW. Nevertheless, recombining the resistance to HB and CBB in one and the same genotype allows breeders to use them as donors in the disease resistance breeding program of snap *Phaseolus* beans.

Table 2. Bacterial diseases response of snap bean lines

Line, Reg.#	Halo blight				Common bacterial blight				Bacterial wilt		
	Race 1		Race 6		Isolate XB96221		Isolate XB99132		PWI	PSI	MDI
	Leaf	Pod	Leaf	Pod	Leaf	Pod	Leaf	Pod			
943-10	7,0	6,5	7,0	7,0	9,0	9,0	9,0	7,3	5,5	3,0	4,3
943-13	8,0	7,0	8,0	7,0	7,0	9,0	9,0	8,0	3,0	2,0	2,5
952-55	5,0	1,7	3,0	1,7	1,0	5,0	3,0	5,0	7,0	7,0	7,0
952-56	6,0	4,0	3,0	4,0	1,7	6,0	3,7	5,0	6,5	6,0	6,3
952-58	5,0	5,0	3,0	4,0	1,0	3,0	5,0	3,0	7,0	5,0	6,0
952-60	4,0	4,0	4,0	1,0	3,0	3,5	3,0	2,0	7,0	3,0	5,0
952-61	4,0	1,0	4,0	1,0	1,0	4,5	3,0	3,0	7,0	5,0	6,0
952-64	6,0	3,5	4,0	2,5	2,0	4,0	3,0	4,0	7,0	9,0	8,0
952-67	5,0	3,0	5,0	3,0	3,0	6,0	5,0	4,5	9,0	9,0	9,0
952-68	5,0	3,5	5,0	3,0	3,0	4,0	3,0	4,0	7,7	6,3	7,0
952-70-3	3,0	6,5	3,0	2,0	7,0	8,0	9,0	5,5	1,0	1,0	1,0
952-71	5,0	4,5	5,0	2,0	1,0	5,0	4,0	4,0	7,0	7,0	7,0
989-118	7,0	3,0	3,0	3,0	3,0	4,0	3,0	3,0	8,3	8,3	8,3
989-127	3,0	6,0	3,0	4,0	7,0	9,0	9,0	8,0	5,0	4,0	4,5
989-138	3,0	5,0	3,0	4,0	9,0	9,0	9,0	9,0	6,3	4,3	5,3
989-139	3,0	5,0	3,0	3,0	7,0	9,0	9,0	9,0	7,0	4,0	5,5
989-141	3,0	7,0	3,0	4,0	9,0	9,0	9,0	9,0	6,3	3,0	4,7
989-143	3,0	7,0	3,0	4,5	9,0	8,0	7,0	8,0	7,5	6,0	6,8
1028-208	3,0	6,5	3,0	4,0	4,0	9,0	9,0	9,0	2,0	1,0	1,5
1056-227	1,0	4,0	6,0	3,0	9,0	9,0	9,0	8,0	1,0	4,0	2,5
1056-227-1	1,0	3,5	4,0	4,0	9,0	9,0	9,0	9,0	1,0	4,5	2,8
1056-227-3	1,0	3,7	4,0	5,3	9,0	9,0	9,0	9,0	1,0	1,7	1,4
1056-236	7,0	6,0	7,0	3,0	9,0	9,0	9,0	7,5	1,0	3,0	2,0
1056-239	7,0	5,0	7,0	3,0	9,0	9,0	9,0	9,0	3,0	5,5	4,3
1056-252	1,0	5,0	4,0	3,0	9,0	9,0	9,0	9,0	1,0	3,0	2,0
1056-253	5,0	6,5	5,0	4,0	9,0	9,0	9,0	9,0	1,0	1,0	1,0
1056-255	4,0	7,0	4,0	5,5	9,0	9,0	9,0	8,0	1,0	5,0	3,0
1056-255-1	5,0	6,0	5,0	6,0	9,0	9,0	9,0	7,0	1,0	5,0	3,0
1056-257-1	5,0	5,5	5,0	4,5	9,0	9,0	9,0	7,0	1,0	4,0	2,5
1074-263	5,0	5,0	5,0	3,5	7,0	9,0	9,0	9,0	1,0	4,0	2,5
1074-264	5,0	7,0	5,0	6,0	7,0	9,0	9,0	7,0	1,0	1,0	1,0
1074-268	1,0	4,0	3,0	5,5	9,0	9,0	9,0	9,0	1,0	3,0	2,0
Isolate means	***4,3 cd****	***4,9 c***	***4,3 cd***	***3,7 d***	***6,3 b***	***7,5 a***	***7,2 a***	***6,8 ab***	-	-	-
r,% **	***0.092***		***0.276***		***0.892***		***0.894***		***0.668***		
r,% ***	***0.520***	***0.618***	***0.520***	***0.618***	***0.894***	***0.936***	***0.894***	***0.936***			

PWI: Plant wilting index; PSI: Plant stunting index; MDI: Mean disease index; *:Means with the same letter in a column are not statistically significant different from each other according to the Duncan test at P≤0.01; **: Analyses of data correlation for leaf/pod reaction; ***: Analyses of data correlation for isolate /host reaction

Our results were obtained from inoculation tests with *Cff* of confirmed different genetic control for wilting and stunting symptoms of BW (Kiryakov et al., 2010). four lines among the included, had no symptoms of wilting (IP 1056-255, IP 1056-255-1, IP 1056-257-1, IP 1074-263), with having values for stunting between PSI=4.0 and PSI=5.0 (table 2). Line IP 952-60 showed distinguished symptoms of wilting (PWI=7), but no stunting (PSI=3.0). Our long term investigations on common bean reaction to BW reviled that stunting symptoms were more severe during years having humid and cooler seasons, compare to the ones with arid and water stress conditions (unpublished data).

Lines IP 1056-227-1 and IP 1074-263 had resistant reaction to both races of HB along with BW resistance, which makes the valuable sources for combined resistance to both *Psp* and *Cff.*

Table 3. Economic properties of eight green bean lines with multiple bacterial disease resistance

Line, Reg.#	Yield of green pods, g plant^{-1}	Yield of standard pods, %	Pod distribution /cm/	Pod suture string	Pod length, cm	Pod width, cm	Sensory evaluation
IP 952-55	130.3 b*	86.0	23	5	12.1	1.0	2.6
IP 952-58	132.5 b	82.8	24	5	12.2	1.0	2.8
IP 952-60	98.9 a	87.5	19	5	11.8	1.0	2.6
IP 952-61	105.6 a	88.5	20	5	12.0	1.1	2.8
IP 952-68	118.2 ab	86.6	19	3	12.2	1.0	3.3
IP 952-71	132.2 b	85.6	19	0	12.5	1.0	4.3
IP 1056-227-1	148.5 bc	88.2	26	0	13.5	1.5	4.5
IP 1074-263	153,8 c	87.0	25	0	15.3	1.6	4.6

*: Means with the same letter in a column are not statistically significant different from each other according to the Duncan test at P≤0.01

The differential reaction of pods and leaves was confirmed for both race 1 and race 6 of *Psp* in 38% and 25% of the tested lines respectively, but not for the two isolates of *Xap.* This indicated that the reaction of these genotypes might be due to a recombination of different genes controlling the response of different plant parts to bacterial infection and that it was important to obtain resistance to *Psp* in both leaves and pods.

A positive correlation between the genotype leaf (r= 0.892) and pod (r= 0.894) reaction and the virulence of the *X.a.pv. phaseoli* isolates included in the study was obtained. This indicates that in changing the isolate, a change in the reaction is less possible compare to the races of *Psp* that had r=0.520 and r=0.618 for foliage and pods reaction respectively. Latter case imposes the necessity of including various races of the pathogen in the breeding process.

Crop breeding for biotic and abiotic stress resistance should be preceded along with adding favorable economic characteristics, with superior fruit and seed yields, fruit quality, early maturity, suitable plant architecture, etc. required from specific country environment and particular market demands (Singh and Schwartz, 2013). Growth habit of the plants of the selected lines was of *Ia* determinate type, suitable for commercial harvest; 55-65 cm height; favorite plant architecture including thick main stems, acute branch angles and pod distribution in upper half of plant, but long internodes and branches. Three of them (IP 952-71, IP 1056-227-1 and IP 1074-263) showed very good sensory quality, possessing stringless, long and strait green pods, along with superior yield.

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