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RESPONSE OF DIFFERENT BEANS AGAINST COMMON BACTERIAL BLIGHT DISEASE CAUSED BY *XANTHOMONAS AXONOPODIS* PV. *PHASEOLI*

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Abstract

POPOVIC, T., M. STAROVIC, G. ALEKSIC, S. ZIVKOVIC, D. JOSIC, M. IGNJATOV and P. MILOVANOVIC, 2012. Response of different beans against common bacterial blight disease caused by *Xanthomonas axonopodis* pv. *Phaseoli. Bulg. J. Agric. Sci.,* 18: 701-707

Common bacterial blight (CBB) in beans, caused by the bacterium *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), is an economically important disease worldwide which reduces crop yields and seed quality. Since there is no satisfactory chemical control for the disease, the use of resistant cultivars is an important management strategy. Sources of immunity are not yet recognized, but tolerance has been reported in several genetic stocks. The main objective of this study was to determine resistance to CBB on twenty-two local and foreign beans grown in Serbia. Two inoculation methods with *Xap* (spraying and multiple needles) as well as naturally infected plants in field conditions were taken for evaluation. The experiments were conducted in randomized complete blocks with three replications. Reaction to *Xap* was assessed as a diseased leaf area and the disease severity index was calculated. The analysis of variance (ANOVA) for disease ratings of leaf reactions indicated significant interactions between cultivars and lines. Results indicate that none of evaluated beans was immune and was found to be resistant to CBB. HR 45, Oreol and XAN 159, -208, -273 were weakly susceptible; Biser, Dobrudzanski rani, KB 100, -101, Medijana, Naya Nayahit, Panonski gradistanac, Panonski tetovac, and Sremac were susceptible while Balkan, Belko, Dobrudzanski rani 7, Dvadesetica, Galeb, Maksa, Slavonski zutozeleni, and Zlatko were highly susceptible cultivars and lines. The five weakly susceptible can be identified and recommend as possible sources of tolerance in plant breeding program.

Key words: common bacterial blight, bean, disease severity index, susceptibility

Introduction

Bean (*Phaseolus vulgaris* L.) is one of the most important legumes worldwide because of its high commercial value, extensive production, consumer use, and nutrient values. In Serbia, this is traditionally a basic food crop and serves as one of a major plant protein source. Diseases are important constraints affecting bean yields. Among the many diseases affecting beans, common bacterial blight disease (CBB) caused by bacterium *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Vauterin et al. (*Xap*) is one of the most destructive bean disease when environmental conditions are favourable for the pathogen (Zaumeyer and Thomas, 1957; Kiryakov, 1999). Yield losses are into the range of 10 and 40%, depending on the intensity of the dis-

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ease, degree of bean susceptibility and environmental conditions that favor the progress of the disease (Saettler, 1989; Opio et al., 1996). In Serbia, CBB was originally reported by Tesic (1946) and during the past few years has become one of the major limiting factors in bean production (Popovic et al., 2007, 2010; Popovic, 2008).

The best alternatives to managing CBB include use of healthy, pathogen-free seed, crop rotation, and ploughing of infected straw (Suchuster and Coyne, 1981). Planting of bean cultivars resistant to *Xap* is economically and technically the most practical method for effective management of CBB (Coyne et al., 1973; Zapata et al., 1985; Yu et al., 1998; Kiryakov and Genchev, 2000; Miklas et al., 2003; Asensio et al., 2006). High level of cultivar resistance would minimize yield losses, reduce bactericide use and facilitate the use of integrated disease management program (Singh and Munoz, 1999).

Immunity to CBB was not detected in beans, although many lines have shown high resistance (Sherf and MacNab, 1986). Resistance to CBB in common bean has been described as a quantitative trait (QTL) with low to medium heritability (Silva et al., 1989). Some sources of resistance to *Xap* have been reported in *Phaseolus coccineus* (Yu et al., 1998), tepary bean *P. acutifolius* (Yu et al., 1998; Singh and Munoz, 1999) and in common (French) bean, *P. vulgaris* (Schuster et al., 1983). The interspecies cross between *P. vulgaris* and either *P. acutifolius* or *P. coccineus* have frequently been used to transfer the resistance-related traits in common bean breeding programs (Tar'an et al., 2001).

Most commercial cultivars of common and snap bean grown in Serbia are susceptible to CBB (Popovic et al., 2007; Popovic, 2008). Chemical control is ineffective and uneconomical to bean growers, and the disease continues to spread (Popovic, 2008). The results of this study could provide the basis for future breeding program for beans resistant cultivars to Xap.

Materials and Methods

The leaf reaction test of *P. vulgaris* to CBB was performed in field conditions. Plants were tested under artificial inoculation conditions using both methods of spraying and multiple needle as well as natural infected plants. The experimental design was a randomized complete block with three replicates and two-row plot of 2m length within each replicate.

Cultivars and lines evaluated. Twenty-two bean cultivars and lines including common and snap bean were sown in Novi Sad region of Serbia. There were 12 samples of Serbian bean (Balkan, Belko, Biser, Dvadesetica, Galeb, Maksa, Medijana, Panonski gradistanac, Panonski tetovac, Slavonski zutozeleni, Sremac, and Zlatko) and 10 samples of foreign beans from Bulgaria (Oreol, Dobrudzanski rani, Dobrudzanski rani 7, KB 100 and KB 101) and USA (Naya Nayahit, HR 45, XAN 159, XAN 208 and XAN 273) (Table 1).

Inoculum. The bacterium strain X24, determined as *Xap* (Balaž, 1991) was used for both inoculation methods of bean plants. Bacterial inocula were prepared from culture grown on yeast extract-dextrose-calcium carbonate agar (YDC) (Schaad, 1988) at 27°C for 48 h by suspending an amount of culture growth in sterile distilled water adjusted to final concentration of approximately 10^8 CFU mL^{-1}.

Spraying evaluation. Spraying was made in the phase V4, third trifoliate leaf (Hall, 1991) with an atomizer using the method described by Schuster (1955). Intensity of leaf disease was performed at the stage of pod filling - R8 (Hall, 1991), using a scale 0-5 (Stavely, 1985).

Multiple needle evaluation. Inoculation with multiple needle was made in development stage R6, flowering (Hall, 1991) according to the method of Andrus (1948). Fifty developed trifoliate leaves were used for one replication of one bean cultivar or line. Intensity of leaf disease was assessed 20 days after inoculation using a scale 1-9 (CIAT, 1987).

Natural infection evaluation. Natural infection evaluation on bean leaves was carried out at the stage of pod filling - R8 (Hall, 1991), using a scale 0-5 (Stavely, 1985).

Disease evaluation. Reaction to *Xap* was assessed as diseased leaf area (DLA).

The disease was evaluated on the basis of disease severity index (DSI) which was calculated as follows:

∑(number of plants in class x severity class) / total number of plants

Table 1
Analysis of variance for CBB (DSI) in evaluated bean cultivars/lines

Cultivar / line	Spraying	Multiple needle	Natural infection	Classes*
	DSI, % (Duncan test)	DSI, % (Duncan test)	DSI, % (Duncan test)	
Balkan	16.03 a	14.94 ab	13.62 a	HS
Belko	16.26 a	15.64 a	14.79 a	HS
Biser	5.95 f	8.69 e	5.81 e	S
Dobrudzanski rani	7.63 e	10.07 d	7.20 e	S
Dobrudzanski rani 7	9.89 d	12.72 c	8.88 d	HS
Dvadesetica	12.64 c	13.11 c	10.83 bc	HS
Galeb	13.77 b	15.38 ab	14.66 a	HS
HR 45	3.03 ij	2.55 h	1.62 g	WS
KB 100	7.28 e	8.87 e	6.78 e	S
KB 101	5.31 fg	8.45 e	6.15 e	S
Maksa	15.56 a	14.75 ab	11.79 b	HS
Medijana	4.96 fg	8.20 ef	3.76 f	S
Naya Nayahit	4.24 gh	7.46fg	5.77 e	S
Oreol	2.06 j	2.40 h	1.42 g	WS
Panonski gradistanac	5.07 fg	7.25 g	3.95 f	S
Panonski tetovac	7.55 e	9.90 d	6.26 e	S
Slavonski zutozeleni	12.45 c	14.63 b	11.60 b	HS
Sremac	9.93 d	10.19 d	7.27 e	S
Xan 159	1.93 j	2.37 h	1.49 g	WS
Xan 208	2.10 j	2.98 h	1.61 g	WS
Xan 273	3.38 hi	3.19 h	1.69 g	WS
Zlatko	12.57 c	12.97 c	9.94 cd	HS
-	$LSD_{0.05}$ = 1.06	$LSD_{0.05}$ = 0.83	$LSD_{0.05}$ = 1.52	-

*Classes: WS = weakly susceptible; S = susceptible; HS = highly susceptible

Statistical analysis. The data were statistically evaluated by analyses of variance (ANOVA). Duncan's multiple range test (LSD) test at the threshold significance of 5% were used to separate treatment means. COSTAT computer software was used for statistical analyses.

Cultivars and lines comparison. Plants presenting grade "no symptoms" were considered as resistant, whereas plants with other grades were considered as susceptible into three susceptibility groupings (weak susceptible, susceptible and highly susceptible) based on the average disease severity rating.

Results

The characteristic symptoms of CBB were observed on inoculated and non-inoculated bean plants. Significant differences in CBB symptoms were observed between bean cultivars and lines in all experiments. All evaluated cultivars and lines showed various levels of susceptibility to *Xap* strain (Table 1, Figure 1). Analysis of variance (ANOVA) for disease ratings of leaf reactions indicate significant interactions between them (Table 1, Figure 1). None of the evaluated cultivar and/or line was immune or found to be resistant to CBB.

Spraying evaluation. DSI of the tested bean cultivars and lines varied between 1.93 and 16.26% (Table 1, Figure 1). XAN 159, Oreol, XAN 208, HR 45 and XAN 273 showed weak susceptibility to CBB with DSI values ranging between 1.93 and 3.38%. Most cultivars and lines (Naya Nayahit, Medijana, Panonski gradištanac, KB 101, Biser, KB 100, Panonski tetovac, Dobrudzanski rani, Dobrudzanski rani 7 and Sremac) were categorized as susceptible with DSI values between 4.24 and 9.93%. Highly susceptible cultivars

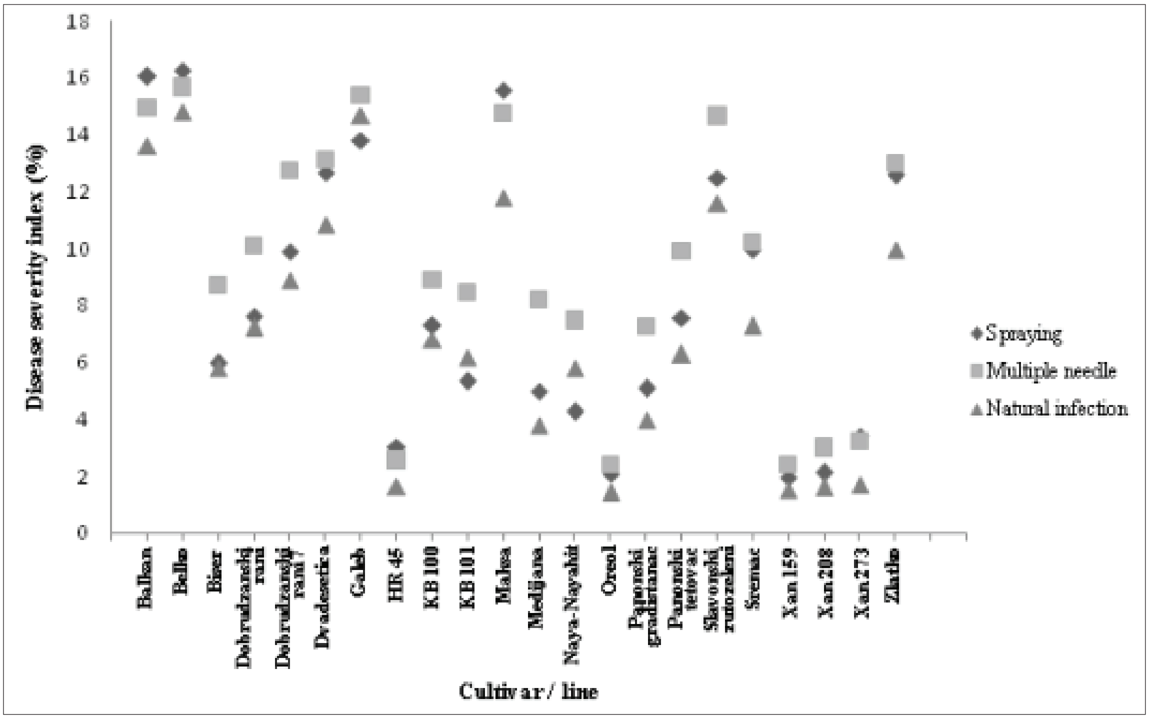

Fig. 1. Reaction of evaluated bean cultivars/lines to *Xanthomonas axonopodis* pv. *phaseoli*

were Slavonski zutozeleni, Zlatko, Dvadesetica, Galeb, Maksa, Balkan and Belko with DSI values between 12.45-16.26%.

Multiple needle evaluation. DSI values of the tested bean cultivars and lines ranged between 2.37 and 15.64% (Table 1, Figure 1). Weak susceptible to CBB were XAN 159, Oreol, HR 45, XAN 208 and XAN with DSI values between 2.37-3.19%. Panonski gradistanac, Naya Nayahit, Medijana, KB 101, Biser, KB 100, Panonski tetovac, Dobrudzanski rani and Sremac were susceptible with DSI values between 7.25 and 10.19%. Highly susceptibility with DSI values ranging from 12.72 to 15.64% was detected in Dobrudzanski rani 7, Zlatko, Dvadesetica, Slavonski zutozeleni, Maksa, Balkan, Galeb and Belko.

Natural infection evaluation. DSI values of the evaluated bean cultivars and lines varied between 1.42 and 14.79% (Table 1, Figure 1). Oreol, XAN 159, -208, HR 45 and XAN 273 showed weak susceptibility to CBB with DSI values ranging between 1.42 and 1.69%. Medijana, Panonski gradistanac, Naya Nayahit, Biser, KB 101, Panonski tetovac, KB 100, Dobrudzanski rani and Sremac with DSI ranging between 3.76 and 7.27% were susceptible. Highly susceptible cultivars were Dobrudzanski rani 7, Zlatko, Dvadesetica, Slavonski zutozeleni, Maksa, Balkan, Galeb and Belko with DSI ranging between 8.88 and 14.79%.

Cultivars and lines comparison. In general, HR 45, Oreol and XAN 159, -208, -273 showed weak susceptibility to CBB; Biser, Dobrudzanski rani, KB 100, -101, Medijana, Naya Nayahit, Panonski gradistanac, Panonski tetovac, and Sremac were susceptible to CBB; Balkan, Belko, Dobrudzanski rani 7, Dvadesetica, Galeb, Maksa, Slavonski zutozeleni and Zlatko were highly susceptible to CBB (Table 1).

The data obtained from artificially inoculated plants and from plants exposed to natural infection experiments agreed (Table 1, Figure 1). These results indicate that the bacterium *Xap* is widespread on commercial beans in Serbia. The relative performance of multiple needles and spraying inoculation techniques did not change or interact significantly, suggesting that both are reliable inoculation techniques for discriminating between susceptibility of bean cultivars and lines.

Discussion

Growing of resistant bean cultivars is one of the the most important measure in controlling CBB. Many researchers worldwide are engaged in breeding program with aim of finding sources of resistance and the production of cultivars resistant to CBB pathogen. Some varieties of bean have a degree of tolerance to *Xap*, but total immunity has not been detected (Sherf and MacNab, 1986).

In Serbia there are insufficient data regarding the susceptibility and/or resistance of most locally grown beans to *Xap*. Previous studies reported that few tepary and common bean genotypes could be sources of resistance genes to *Xap* (Schuster et al., 1983; Park et al., 1998). The objective of this study was to evaluate some existing local and foreign *P. vulgaris* for resistance to CBB. The five weak susceptible cultivars could be used as possible sources of CBB tolerance. All evaluated cultivars and lines had visible lesions on their leaves in all experiments, and therefore none were considered as either immune or resistant to CBB. The summary of the analysis of variance for the experiment on artificial inoculation and natural infection of CBB (DSI) showed that there was no significant difference between cultivars / lines and the source of infection. This means that a cultivar or a line found to be susceptible when artificially inoculated was also susceptible under natural infection. These results indicate that the bacterium *Xap* is widespread on beans in Serbia because of the high level of naturally infected plants.

There is evidence that CBB resistance can be incorporated from lines into bean cultivars in common bean breeding programs. Resistant common bean germplasm line HR45 was developed by Park and Dhanvantari (1994) and navy bean cv. OAC Rex by Michaels *et al*. (2006). Three Great Northern types named Tara, Valley and Jules had a moderate (Tara and Valley) and high (Jules) degree of tolerance. Other sources include Ruse 6 (Bulgaria), Grisbeck, Green Plentiful, Medal Refugee and Goliath Waxy (Russia). High level of tolerance was found in PI lines of *P. vulgaris* (in Turkey PI 169727 and PI 167399, in Colombia PI 207262, in Mexico PI 197687, in India PI 163117), Guali (ICA, Colombia), Great Northern Nebrasca#1 sel. 27 and Barteldes Lima (Coyne and Schuster, 1973). *P. coccineus* and QTL marker as a source of resistance are held in the following: ICB-3, -6, -8, USPT-CBB-1, -2, -3, USBK-CBB-5, USNA-CBB-1, -2, -3, -4, USGN-CBB-4, USCR-CBB-12, -13, USLK-CBB-9, -10 (Miklas et al., 1999, 2001a, 2001b, 2001c), XAN 112, -309 (CIAT), Chase, Weihing (Coyne et al., 1994, 2000), BelNeb-RR-1 (Stavely et al., 1989), W-BB-11, -20-1, -35, -52, -11-56 (Zapata et al., 2004); VAX 3, -5, -6 (Singh et al., 2001), ABCP-8 (Mutlu et al. 2005), Wilk-2, CBB-Teebus (Fourie and Herselman, 2002), PR9443-4 and Pomjor 17 (Beaver et al., 1992, 1999).

To conclude, we found that screening bean cultivars and lines for *Xap* tolerance in the field experiments using two methods either of artificial inoculation or under the natural infection conditions enabled us to identify five weak susceptible. The significance of this study is in presenting the potential sources of tolerance to CBB in plant breeding programs.

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