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Evaluation of Dry Bean Cultivars in Turkey for Resistance against Common Bacterial Blight Disease

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ABSTRACT

Background: Common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) has been recorded as one of the most important seed-borne destructive diseases of beans, affecting its production worldwide. Since chemical compounds cause serious damage to natural ecosystems and in some instances fail in controlling CBB, the use of genetically resistant cultivar is the most economically efficient and environmentally friendly measure in the control of CBB disease. The objective of this study was to assess the resistance reactions of widely grown and popular common bean cultivars against Xap under controlled conditions.

Methods: In this study, 16 dry bean cultivars comprising eight widely grown dry bean varieties, six improved and promising genotypes and two known CBB susceptible varieties, were evaluated for their reaction to the CBB in a completely randomized design with three replications. The reaction of the bean cultivars to Xap was assessed using disease incidence, disease severity, per cent severity index, the area under disease progress curve (AUDPC) and incubation period.

Result: Data analysis indicated that there were highly significant differences ($p \leq 0.01$) among common bean cultivars for CBB disease incidence, disease severity, per cent severity index and AUDPC among cultivars. The cultivated varieties Noyanbey and Özmen were identified as resistant cultivars. In addition, SAP6 and BAC6 resistance genes were found in these two cultivars. As a consequence, it was thought that these cultivars could be a potential source of disease resistance for CBB in breeding programs and sustainable, eco-friendly and good agricultural practices.

Key words: Bacterial blight, Bean cultivars, Disease resistance, Xap.

INTRODUCTION

Bean (*Phaseolus vulgaris* L.), a highly produced legume crop in the world, is one of the most important crops nutritionally and economically (FAO, 2016). Dry beans contain high levels of high-quality proteins and are an excellent source of complex carbohydrates, such as fiber, starch and oligosaccharides, as well as important vitamins and minerals and are recommended as a meat alternative in the human diet (Ceyhan *et al.,* 2014; FAO, 2016).

CBB caused by the gram-negative bacterial pathogen, Xap and its fuscans variant *X. a.* pv. *phaseoli* var. *fuscans* (Xapf) have been recorded as one of the most important seed-borne destructive diseases on beans worldwide. It is a serious and critical bacterial disease of common bean which causes lesions on the leaves, stems, pods and seeds of the plant. It occurs at any stage of plant development, affects seed quality and results in yield losses of 20-60% and finally leads to economic losses of thousands of millions of US dollars (Belete and Bastas, 2017).

CBB is the most destructive bacterial disease that causes a reduction of yield and quality of bean production in Turkey (Donmez *et al*., 2013). It is generally endemic in bean growing areas under favorable environmental conditions such as high temperature, rainfall and relative humidity) (Dursun *et al.,* 2002). Since breeding for host plant resistance is reported as the most effective and long-term measure to control the disease, plant breeders and pathologists in different countries and organizations are working together to identify resistance reaction of the different bean genotypes using single or multiple races of Xap to develop and identify broad resistance to CBB (Zapata *et al*., 2011; Singh and Miklas, 2015).

Chemical pesticides which are incessantly used on beans to manage CBB disease cause serious damage to agricultural and natural ecosystems and they showed frequent failure for controlling CBB. Since there is no satisfactory chemical control against Xap, the use of resistant varieties is an important management strategy (Zapata *et al*., 2011).

According to earlier research results, bean cultivars were not found to be resistant to CBB in Turkey (Benlioğlu *et al.,* 1994). However, Dursun *et al.* (2002) screened 22

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genotypes in greenhouse for resistance against Xap strain and one genotype (AG-7117) was found resistant to CBB. This was the first resistance source report in common bean line against Xap in Turkey. Later, Donmez *et al.* (2013) conducted a greenhouse and field experiment to screen 38 bean genotypes against Xap and only one genotype (36K) was found to be resistant to the pathogen. The objective of this study was to assess the resistance reactions of widely grown and popular common bean cultivars against Xap under controlled conditions.

MATERIALS AND METHODS

The experiment was conducted during 2018-19 under greenhouse conditions at Selcuk University, Turkey. Sixteen dry bean cultivars/genotypes comprising eight known and widely grown dry bean cultivars (Akman, Alberto, Gereat × Northan, Göynük, Kınalı, Noyanbey, Özmen and Şehirali), six promising lines (PV042, Şeker Fasulyesi, Büyük Fasulye, PV1 × Cocinous, PV12 × Akman and PV12 × Alberto) and two known susceptible varieties (Aras 98 and Mexican-142) were selected and evaluated for their reaction to Xap pathogen under greenhouse conditions. Highly virulent which was biochemically and molecularly characterized Xap isolate was obtained from Selcuk University and grown on nutrient agar medium for 48 hours at 28°C. Bacterial suspension was prepared and the concentration was adjusted to 10^8 CFU ml^{-1} in sterile distilled water (SDW) using a spectrophotometer.

The treatments were replicated thrice in a completely randomized design and repeated twice. Standard size plastic pots were filled with sterilized mix (1:1:1, soil, peat and burnt manure). Seeds of the different common bean cultivars were sterilized in 10% sodium hypochlorite for 3 minutes and washed with SDW twice. After emergence, the plants were watered as required and the greenhouse temperature was maintained at 25°C±2 throughout, with 16 h light and 8 h dark. At three-leaf stage all plants were ready for bacterial inoculation. Xap suspension was sprayed on the aerial part of fully expanded leaves using a pressure handheld sprayer. Similarly, control plants were also sprayed with SDW. The greenhouse cabinet was covered by its transparent nylon material for three days after inoculation to create needed humidity for bacterial development (Dursun *et al.*, 2002). To fulfill the Koch postulates, re-isolation of the Xap from the inoculated plants was done at the end of the experiment based on the procedure by Bradbury (1970). The re-isolate Xap was confirmed based on the physiological, biochemical and molecular (using X4c/X4e primers) tests (Bastas and Sahin, 2017). Measuring of disease incidence and severity commenced after 14 days of Xap inoculation and repeated every 7 days over four periods. The first date on which CBB symptom was observed was recorded and the incubation period was determined (Dursun *et al.* 2002; Donmez *et al.* 2013). The CBB disease severity was assessed on a 0-9 visual scale as modified (Fig 1); where 0 = no infection, 1 = 1%, 2 = 2-5%, 3 = 6-10%, 4 = 11-15%, 5 = 16-30%, 6 = 31-50%, 7 = 51-75%, 8 = 75-85% and 9 = >85% lesion area on the infected leaves (CIAT, 1998; Ararsa *et al.,* 2018). Disease susceptibility was determined based on severity score or average disease severity rating [Immune (0), highly resistant (≤1), resistant (≤3), moderately susceptible (≤5), susceptible (≤7) and highly susceptible (> 7)] (CIAT, 1998; Ararsa *et al.* 2018).

Disease incidence (DI) was calculated based on the number of leaves that showed CBB relative to the total number of leaves of each plant.

$$DI = \frac{\text{Number of leaves}}{\text{Total number of leaves observed}} \times 100$$

The severity grade was converted into percentage severity index (PSI) using the following formula:

$$PSI = \frac{\text{Total numbers of rating}}{\text{Total plants assesed} \times \text{Maximum rating (score) in scale}} \times 100$$

The area under the disease progress curve (AUDPC) was calculated from the PSI values using the following formula:

$$AUDPC = \sum_{i=1}^{n=1} \left[\left(\frac{Xi + Xi + 1}{2} \right) \times (ti + 1 - ti) \right]$$

Where

Xi = Per cent severity index expressed as a proportion at the i^{th} observation,

ti = The time (days after planting/inoculation) at the i^{th} observation.

n = The total number of observations. Its values were expressed in the form of %-days.

After screening the bean cultivars against Xap under greenhouse, PCR amplification for Sequence Characterized Amplified Region (SCAR) markers (SAP6, BAC6, BC420 and R7313) from genomic DNA were performed and scanned to detect and confirm the presence of resistance genes in the two bean cultivars (Noyanbey and Özmen).

Data on disease incidence and severity, incubation period, percept severity index and AUDPC were subjected to analysis of variance (ANOVA) with SAS 9.1.3 computer software. Where ANOVA detected significant differences among treatments ($p<0.05$), treatment means were separated using Tukey multiple range tests.

RESULTS AND DISCUSSION

Evaluation of cultivars is very important in the common bean improvement program, to identify sources of disease resistance by incorporating different agronomic traits like yield, early maturity, growth habit and other yield-related traits which are positively correlated to yield. This helps in facilitating the development of superior varieties for small and large-scale production sector (Alladassi *et al.*, 2018).

The analysis of variance indicates that there were highly significant differences ($p\leq0.01$) among common bean cultivars for CBB disease score, PSI and AUDPC (Table 1). For disease incubation period, there was also significant

Table 1: Summary of mean squares for ANOVA of 16 dry bean cultivars inoculated with Xap for all measured variables.

Variables[€]	Cultivars (15)[¥]	Error (32)	CV (%)	R^2
DS	8.542**	0.303	9.74	0.93
PSI	1054.532**	37.508	9.76	0.93
AUDPC	310857.40**	20303.696	14.27	0.88
DI	954.062**	15.580	7.90	0.97
IP	1.617*	0.536	7.48	0.59

[¥] = Numbers in parenthesis represent degrees of freedom.

**, * = Significant at P≤0.01 and significant at P≤0.05.

[€] = Abbreviations: DS= Disease severity, PSI= Per cent severity index, AUDPC= Area under the disease progress curve, DI= Disease incidence, IP= Incubation period.

difference (p≤0.05) among cultivars. The symptom of CBB was observed on the leaves of bean plants after 8-11 days. Overall, results showed that disease scores ranged from 2.39 (Noyanbey) to 7.67 (PV042) (Table 2). A different level of variation among bean genotypes were also observed for different disease measurements and reported by different scholars (Nkhata, 2016). Similarly, Alladassi *et al.* (2018) reported that disease scores ranged from 2.2 to 7.8 for bean leaves based on CIAT disease scale.

In this study, the tested bean cultivars were classified into four disease reaction type based on their severity score 35 days after inoculation (DAI). Consequently, lower DI, shorter incubation period, lower disease infection rate and AUDPC were associated with resistant cultivars, whereas the reverse was true for susceptible bean cultivars. Among the tested genotypes, two of the cultivated varieties (Noyanbey and Özmen) showed resistance reactions to Xap with mean disease severity ratings of 2.39 and 2.73, respectively. The cultivars, Akman, Gereat × Northan and Kınalı showed moderate resistance with mean rating scales ranging between 3.62 and 4.67. Nine genotypes namely Alberto, Mexican-142, Goynuk, Şehirali, Şeker Fasulyesi, Büyük Fasulye, PV1 × Cocinous, PV12 × Akman and PV12 × Alberto were categorized as susceptible (5.33-6.95) and the remaining two cultivars (PV042 and Aras 98) were highly susceptible with average disease severity score of 7.67 and 7.34, respectively (Table 2). Similar finding was reported by Dursun *et al.* (2002), Donmez *et al.* (2013), Ararsa *et al.* (2017) and Alladassi *et al.* (2018).

Similar to disease rating, the minimum disease infection rate was observed in the cultivar Noyanbey (26.55%) and maximum in the most susceptible genotype PV042 (85.19%). Similarly, there was also variation for CBB disease incidence among the cultivars. The average CBB incidence over the disease's assessment periods ranged from 24.29 to 72.47% for the evaluated bean cultivars. Accordingly, lowest DI was observed from the cultivars, Noyanbey and Özmen (24.29 and 27.18%, respectively) and the highest DI from susceptible check cultivar Aras 98 (72.47%) and PV042 (71.25%). 56% of the tested genotypes showed less than 50% disease incidence on their leaves. However, 75% of the cultivars showed more than 50% infection rate (Table 2). Dursun *et al.* (2002), Donmez *et al.* (2013), Ararsa *et al.* (2017) and Alladassi *et al.* (2018) reported similarly which was consistent with our result.

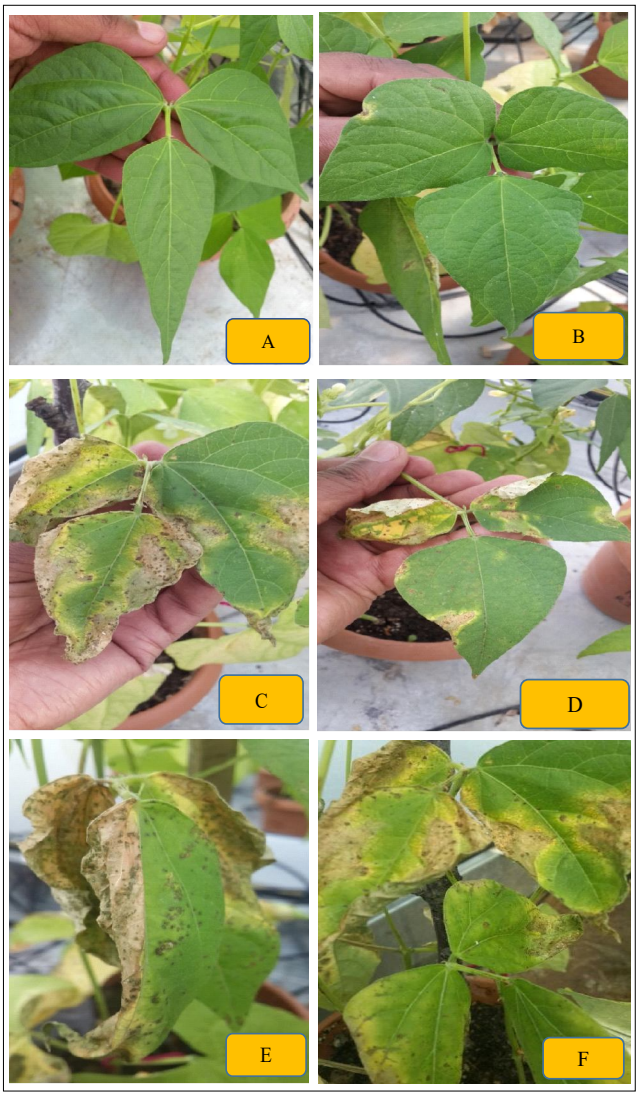

Fig 1: Disease rating scales (0-9) for CBB in dry beans used to identify resistant and susceptible cultivars. A: score 0, B: score 1, C and D: score 3-5 and E and F: score 6-8.

The result obtained showed that there was positive correlation between disease severity and DI, such that those

Table 2: Overall mean of disease severity score, PSI, AUDPC and DI against Xap.

Name of bean cultivars	Disease severity (0-9 scale)	Disease reaction type*	Severity index (%)	AUDPC	Disease incidence (%)	Incubation period
Akman	4.67	MR	51.85de	756.20efg	30.60fg	11.00a
Alberto	5.61	S	62.35cd	898.81def	37.70e	10.50abc
Mexican-142	6.61	S	73.46b	1246.64ab	69.72ab	9.17cde
Gereat × Northan	3.62	MR	40.13fg	566.10gh	29.35g	10.33abcd
Göynük	6.56	S	72.84bc	1153.75abcd	46.65d	9.33bcde
Kınalı	3.95	MR	43.83ef	663.29fgh	37.10ef	9.33bcde
Noyanbey	2.39	R	26.55h	468.85h	24.29g	10.67ab
Özmen	2.73	R	30.25gh	481.86h	27.18g	11.00a
Şehirali	5.33	S	59.26d	933.38cde	42.83de	9.67abcde
PV042	7.67	HS	85.19a	1406.53a	71.25ab	9.50bcde
Şeker Fasulyesi	6.95	S	77.16ab	1266.10ab	70.78ab	9.17cde
Büyük Fasulye	6.95	S	77.16ab	1220.73ab	64.32bc	9.83abcde
PV1 × Cocinous	6.73	S	74.69ab	1181.84abc	59.81c	9.83abcde
PV12 × Akman	6.39	S	70.99bc	1084.63bcd	47.06d	9.83abcde
PV12 × Alberto	6.95	S	77.16ab	1268.24ab	67.85ab	9.00de
Aras 98	7.34	HS	81.49ab	1382.77a	72.47a	8.50e

Means followed by the same letter within a column are not significantly different from each other at P≤0.05 according to Tukey.

* = Abbreviation: R= Resistant; MR= Moderately resistant; S= Susceptible; HS= Highly susceptible; AUDPC= Area under the disease progress curve.

cultivars that showed resistant reaction had low DI and vice versa. For example, the most susceptible cultivar PV042 had a 7.67 disease severity rating value with 71.25% of DI whereas the cultivar Noyanbey with 2.39 rating scale had 24.29% DI. Similarly, cultivars that showed highest severity score and PSI had the highest disease progress rate or AUDPC (Table 2). The relative AUDPC ranged from 468.85 in Noyanbey to 1406.53%-day in PV042 with 43.75% of cultivars having AUDPC of less than 1000 and the rest (56.25%) between 1084.63 and 1406.53%-day. Alladassi *et al.* (2018) reported that disease scores ranged from 2.2 to 7.8 for bean leaves based on CIAT disease scale.

The incubation period ranged from 8.5 to 11 days. The shortest incubation period was observed in the cultivar Aras 98 followed by PV12 × Alberto and the longest incubation period was observed in cultivar Akman and Özmen (11 days). Likewise, Kassahun (2008) observed highly significant differences among the bean cultivars in relation to incubation period.

The PSI over different time intervals revealed that there was a progress in the infection rate starting from 14th days after inoculation to 35th days after inoculation. However, there was no significant difference during the 28th and 35th days after inoculation for each respective cultivar. The infection rate was almost constant after 28 days of Xap inoculation (Table 3). Varieties, Aras 98, Mexican-142, Şeker Fasulyesi and PV12 ×Alberto were highly affected (40.74, 39.51, 36.42 and 33.33%, respectively) compared to others at day 14 whereas Akman, Gereat × Northan, Özmen, Alberto, Noyanbey and Kınalı were the least affected with an infection rate not more than 20% on this particular day (Table 3). The infection rate of CBB on cultivar Akman was very low on the first day of data collection (9.88%) performing better than the two resistant cultivars (Noyanbey and Özmen). However, with time, this cultivar became moderately susceptible. The same was true for the susceptible cultivar, Alberto which was less affected (12.96%) on day 14 but was subsequently categorized as being among the most susceptible cultivar at the end of day 35. This indicates that some cultivars were resistant in the early period of disease development but become more susceptible when disease develops over. Therefore, for such cultivars, early disease management or control measure is more crucial hence recommended.

As observed from Table 3, the transition period from days 21 to 28 was widespread. At day 21, about 10 (63%) of the cultivars had less than 50% infection whereas during the same period (21 DAI), 11 (69%) cultivars had more than 50% infection rate. Previously, similar research results were also reported in Turkey and in different parts of the world for differentiating the interaction between different bean genotypes with CBB pathogen and identifying some potential genotypes which had a resistant gene for CBB resistance breeding activities (Dursun *et al.*, (2002; Donmez *et al.*, 2013; Ararsa *et al.*, 2017; Alladassi *et al.*, 2018). On the contrary, Benlioğlu *et al.* (1994) and Takudzwa *et al.* (2017) showed that none of the evaluated beans were immune and resistant to CBB in Turkey and Serbia, respectively.

After screening of the bean cultivars against Xap, the presence of resistance genes was detected only in Noyanbey and Özmen cultivars. With the SCAR markers, 820 bp band was obtained for SAP6 and 1250 bp band for BAC6 in these two cultivars with PCR amplification. However,

Table 3: Mean PSI of bean cultivars in relation to CBB.

Cultivars	PSI			
	14 DAI	21 DAI	28 DAI	35 DAI
Akman	9.88f	32.10def	45.06c	51.85de
Alberto	12.96def	40.74bc	50.00c	62.35cd
Mexican-142	39.51ab	52.47abc	69.14b	73.46b
Gereat × Northan	10.50ef	23.46ef	32.10de	40.13fg
Göynük	25.93abcdef	49.38abc	66.05b	72.84bc
Kınalı	18.52cdef	24.07def	39.51cd	43.83ef
Noyanbey	14.82def	20.99f	25.31e	26.55h
Özmen	11.11ef	20.99f	27.16e	30.25gh
Şehirali	25.93abcdef	38.89cde	51.86c	59.26d
PV042	26.54abcde	62.35a	82.72a	85.19a
Şeker Fasulyesi	36.42ab	52.47abc	71.61ab	77.16ab
Büyük Fasulye	28.40abcd	49.39abc	72.22ab	77.16ab
PV1 × Cocinous	23.46bcdef	50.00abc	69.75b	74.69ab
PV12 × Akman	25.31abcdef	40.13cde	66.67b	70.99bc
PV12 × Alberto	33.33abc	54.32abc	71.61ab	77.16ab
Aras 98	40.74a	58.03ab	78.40ab	81.49ab

Means followed by the same letter within a column are not significantly different from each other at P≤0.05 according to Tukey. DAI= Days after inoculation, PSI = Present severity index.

we did not obtain any result for resistance genes by BC420 and R7313 markers in analyzed bean cultivars. Similarly, Poyraz *et al.* (2017) screened ten resistance genes against Xap and Psp in 12 local bean cultivars using molecular markers (SCAR). They found that there were a presence/ absence of the tested resistance genes in bean varieties against Xap and Psp.

CONCLUSION

Developing genetically resistant cultivar is the most economically efficient, ecologically friendly and socially acceptable in the control of CBB disease. Most of the bean cultivars used in the study area showed susceptible reaction to Xap except the two varieties (Noyanbey and Özmen). Therefore, these two cultivars can be a potential source to CBB resistance in plant breeding programs. They can be utilized in bean crop improvement program to introgress resistance into susceptible bean cultivars alongside other agronomic traits. Further studies should be conducted under field conditions using large number of samples in different environmental conditions and using different Xap strains from different parts of the country to further assure the quality of resistance exhibited by the two resistant cultivars.

Conflict of interest: None.

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