ORIGINAL ARTICLE

Evaluation of Common Bean Cultivars for their Resistance Against *Xanthomonas Axonopodis Pv. Phaseoli*

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ABSTRACT

Common bacterial blight (CBB) caused by the bacterium *Xanthomonas axonopodis* pv. *phaseoli* (Xap), is an economically important disease of beans worldwide. Since there was no satisfactory chemical control for the disease, use of resistant cultivars is an important management strategy. The main objective of this study was to determine CBB reaction of twenty different bean genotypes breed and/or maintained by Melkassa Agricultural Research Center. The genotypes were evaluated under field and greenhouse conditions for their reaction to Xap following leaf-spray inoculation with bacterial suspension of 10^8 cfu/ml. Disease was ratted with 0 - 9 visual scale and the percent severity index (PSI) was calculated. The results revealed that the evaluated genotypes showed various level of reaction to Xap. Three genotypes GLP-2, ECAB005 and Gobe Rasha were showed resistant reaction against Xap. Eleven genotypes showed moderately susceptible disease reaction with mean disease severity rating values ranging from 3.57- 5.00 while six genotypes were categorized as susceptible and one genotype Mexcan-142 showed high susceptibility. The study results were in a conclusion that there is a resistant gene in the resistant and moderately susceptible genotypes. Therefore, the resistant genotypes should be promoted in bean production system to increase common bean production and productivity in reducing CBB epidemics and associated yield loss. Moreover, the resistant and moderately susceptible genotypes identified can be used as possible sources of resistance for breeding program against CBB to improve the susceptibility of commercial bean cultivars commonly cultivated in the study area.

Key words: bean susceptibility, common bacterial blight, disease ratting, Disease resistance

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.), one of the most important crops in terms of both economy and nutrition, is cultivated in almost all regional states of Ethiopia. The production and productivities of the crop vary from area to area and productivity is generally low (ICARSAT, 2011). The main yield constraints are fungal, viral and bacterial diseases (Tadesse *et al*., 2008). Among bacterial diseases, common bacterial blight (CBB) is one of the most distractive disease and reported to cause yield loss up to 21 % (Fininsa, 2001) in eastern Ethiopia.

Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (syn. *Xanthomonas campestris* pv. *phaseoli*) and its fuscous variant (var.) fuscans is also one of the most destructive diseases of common bean, globally and cause significant yield loss in susceptible cultivars (Mutlu et al. 2005b). Reported yield loss ranges from 10 to 40% (Mutlu *et al*., 2005a). The amount of yield loss depends on the intensity of the disease, environmental conditions that favor the onset and progress of the disease, and the degree of susceptibility of the cultivars (Asensio *et al*., 2006). The pathogen is a major seed-borne disease of common bean worldwide (Tar'an *et al*., 2001; Miklas *et al*., 2003) and also overwinter in the soil with infected debris (Fininsa and Yuen, 2002). Furthermore, the ability of *X. campestris* pv. *phaseoli* epiphytic populations to survive on non-host weed plants (Gent *et al*., 2005) makes the pathogen difficult to control using disease free seed. Chemical control is ineffective and uneconomical to bean growers, and the disease continues to spread (Popovic, 2008). Moreover if the pathogen is endemic in a region, conducive weather conditions such as warm temperatures and wind-driven rains can facilitate the spread of CBB bacteria from these local inoculum sources, leading to disease development in fields of susceptible cultivars established with pathogen-free seed. Thus, genetic resistance is the most effective method of control against Xap (Miklas *et al.* 2003). Therefore, the incorporation of adequate levels of CBB resistance into common bean cultivars is an important component of an integrated disease management program.

The search for sources of resistance in *P. vulgaris* has led to the identification of partial resistance, which is usually found to be quantitatively inherited (Beebe, 1989). However, useful sources of resistance to CBB have been found in the related species *P. coccineus* (Welsh and Grafton, 2001) and *P. acutifolius* (Marquez *et al*., 2007). Interspecies crosses 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). Despite the complexity, these resistance traits have been introduced into common bean breeding lines. However, most of these original and derived sources of resistance are poorly adapted in tropical conditions (Silva *et al*., 1989).

In Ethiopian condition, both in regional and national research system, the established common bean nursery have been the basic activities for host plant resistance development program and different resistance materials are released and still some are under production (MoARD, 2014). Constraints in resistance breeding were susceptibility of the resistant cultivars to the virulent races (pathotypes) in another area (CIAT, 1999). Moreover, CBB resistance is also reported to be associated with plant architecture, indeterminate growth habit and late maturity (Tar'an *et al*., 2001). Thus, periodic evaluation of cultivars is very important to continually make use of the resistant cultivars in breeding program for disease resistance. Therefore, identification of any cultivars with resistance to CBB pathogen among the cultivars grown in the country would be of great benefit to reduce the CBB epidemic development in the country by using the resistant cultivars as a

component of integrated disease management strategy. Thus the objective of the present study was to evaluate response of 20 different bean genotypes breed and/or maintained by Melkassa Agricultural Research Center under both greenhouse and field conditions in order to identify those with resistance reaction to CBB pathogen.

MATERIAL AND METHODS

Description of the study area

The experiment was carried out during 2015 main growing seasons at two sites in the central rift valley area namely Melkassa Agricultural Research Center (Melkassa) and Arsi Negele Agricultural Research Sub station (Arsi Negele). Melkassa is located 99 km southeast of Addis Ababa in the semi-arid region of Central Rift Valley at 8°24′ N latitude, 39° 12′ E longitude and the altitude of the area is 1550 m.a.s.l. The ten years (2003 to 2012) average weather data show that the area receives an average of 915.7 mm annual rainfall and the maximum and minimum annual mean temperatures are 28.9°C and 13.8°C, respectively. The soil type of the site is Andosol which is cultivated for long period of time (MARC, 1997).

Arsi Negele is also one of the sub-centers of MARC and located to 228 km south of Addis Ababa at 7° 25′ N latitude, 38° 31′ E longitude and an elevation of 1900 m.a.s.l**.** The past ten years (2003 to 2012) data shows the area receives an average annual rainfall of 881.2 mm and the maximum and minimum annual mean temperatures of 27°C and 10.6°C, respectively. The soil type of the site is Nitosol (MARC, 1997).

Experimental material and treatments

Planting material: Twenty introduced and/or local breed commercial common bean cultivars from Melkassa Agricultural Research Center were evaluated for their reaction to common bacterial blight pathogen in both green house and field. Names and characteristics of these cultivars are provided in Table 1.

Inoculum preparation: Xap isolates collected from different locality of central rift valley were used to prepare inoculum. Isolates for inoculation were cultured on YDCA (Yeast extract Dextrose Calcium Carbonate Agar) for 48h at 28°C. The bacterial colonies were transferred in to flasks containing liquid medium (Nutrient Broth) and incubated at shaker with 150 rpm for 24 hours. Then the bacterial cell suspension was adjusted approximately to 10^8CFU/ml.

Experimental design and management

Greenhouse experiment: A pot experiment was conducted under greenhouse conditions to evaluate bean cultivars for resistance to Xap. Twenty-centimeter pots were filled with sterilized soil, sand and compost in the ratio 4:1:1 and planted with five seed in each pot. Three plants were maintained in each pot after germination. The experiment was arranged in the greenhouse in completely randomized block design with three replications. Trials were irrigated prior to inoculation and bacterial suspension was sprayed on plants at the age of 21 days after planting with hand sprayer. The floor of the greenhouse was covered with fiber sucks and kept wet to generate humidity in order to favor development of CBB. Each trial was inoculated in late afternoon and repeated weekly for three times to enhance disease development. Disease observation was done starting from 14days after the first inoculation and thenafter every two weeks until maturity.

Table 1: Name and characteristics of bean cultivars used for evaluation

No	Official Name	Local Name	Year of release	Growth habit	Day To Maturity	Source Name	Constraints_1 addressed	Constraints_2 addressed	Constraints_3 addressed	Constraints_4 addressed	Gene Pool
1	Dicta-105	Nasir	2003	Bush	85-100	CIAT line	Drought	CBB	HB		Meso American
2	DOR-554	Dimtu	2003	Bush	85-100	CIAT line	CBB				Meso American
3	XAN-310	Dinknesh	2006	Bush	85-90	CIAT line	Drought	CBB	HB		Meso American
4	RAB-484	Melka Dima	2006	Bush	85-91	CIAT line	Drought	CBB	HB		Andean
5	STTT-165-92	Chore	2006	Bush		CIAT line	Yield	CBB	HB	Anthracnose	Meso American
6	ACOS Red	Acos red	2007	Bush	70-80	North America	CBB	Rust			Andean
7	SUG131	Deme	2008	Bush	85	CIAT line	CBB	HB	Anthracnose	ALS	Andean
8	Batu	Batu	2008	Bush	75-85	MARC cross	Drought	CBB	HB		Andean
9	GLP-2	GLP-2	2011	Bush	84	NARS (KARI)	Drought	HB	CBB	Adaptation	Andean
10	ECAB-0056	ECAB	2012	Bush	85	CIAT line	CBB	HB	Anthracnose	ALS	Andean

11	STTT-165-96	Chercher	2006	Bush	95-105	CIAT line		Yield	ALS	Meso American
12	G-2816	Gofta	1997	Bush(indeterminate)	90-100		Rust	Anthracnose		
13	GLPX 92	Ayenew	1997	Bush(indeterminate)	90-100		Rust	Anthracnose		
14		Melkie								
15	AFR-772	Ibado	2003	Bush(indeterminate)	90-120		CBB	Rust	HB	
16	G4445	Awash-1	1990	Bush(indeterminate)						
17	PAN-182	Awash Melka	1999							
18	Roba-1	Roba	1990							
19	ICS-15541	Gobe Rasha	1999							
20	G11239	Mexcan-142	1973	Bush	90					

Field experiment: Field experiment for the evaluation of cultivars reaction to Xap was conducted at two locations (Melkassa and Arsi Negele trial site) of Melkassa Agricultural Research Center in 2015 main cropping season with the same cultivars used in greenhouse experiment. The experiment was laid in Randomized complete Block Design (RCBD) with three replications and a plot of 2 m × 2 m used as treatment plot. Each treatment was separated by 1m between plots and 2 m between blocks. Inoculation was made on 21days aged plant (at age of first trifoliate leaf). Plants were sprayed with water prior to inoculation in order to provide a favorable microclimate for bacterial infection. Each trial was inoculated in the late afternoon with knapsack sprayer and inoculation was repeated three times at week interval to enhance disease development. Plants were rated for disease reaction 14 days after the first inoculation and thenafter assessed every two weeks until maturity.

Data collection

Leaf disease severity was assessed as modified CIAT 0 - 9 visual scale 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). Disease severity was assessed on all the three plants in case of greenhouse experiment and 10 randomly selected and per tagged plants per plot in field experiments. The final mean disease assessment score was used to group cultivars in to six disease reaction classes as 0 = immune, ≤1= highly resistant, ≤ 3= resistant, ≤ 5 moderately susceptible, ≤ 7 = susceptible and ≥ 7 = highly susceptible. Percent severity index (PSI), Area Under Disease Progress curve and disease progress rate was calculated from disease severity data to see the nature of disease development during the epidemic period.

Data analysis

Mean disease severity, Area Under Disease Progress Curve (AUDPC) and disease progress rate data were subjected to analysis of variance with SAS 9.2 computer software GLM procedure. Where ANOVA detected significant differences among treatments ($p<0.05$), treatment means were separated using the Least Significant Difference (lsd).

RESULTS AND DISCUSSION

Disease severity rating result showed that there was a difference in CBB disease reaction between common bean cultivars in both greenhouse and field experiments (Table 2). None of the evaluated cultivars were immune but three of the common bean genotypes (ECAB0056, GLP-2 and Gobe Rasha) shows a resistant reaction to Xap with mean disease severity rating values ranging from 1.6 to 2.67. The remaining cultivars were grouped to three susceptibility groups (moderately susceptible, susceptible, and highly susceptible) based on the mean disease severity rating in greenhouse and field experiments (Table 2). The result of both greenhouse and field experiments showed genotypes: Acos Red, Chercher, Chore, Deme, Dimitu, Dinknesh, Ibado, Melka Dima, Melkie and Nasir showed moderately susceptible with mean disease severity rating values ranging from 3.57 to 5.00. Six genotypes (Awash-1, Awash Melka, Ayenew, Batu, Gofta and Roba) were categorized as susceptible in greenhouse and field experiments except Ayenew was highly susceptible under greenhouse evaluation and Awash Melka was moderately susceptible under field evaluation at Melkassa. One genotype Mexcan-142 showed high susceptibility reaction in greenhouse and field trial at Arsi Negele but susceptible at Melkassa (Table 2).

In privies study Kasahun (2008) in his evaluation of twelve bean genotypes under greenhouse condition reported Ayenew and Mexican-142 were

categorized as susceptible; Awash Melka and Roba-1 as moderately susceptible and Gobe Rasha as resistant cultivars. The authors concluded that symptom development in these cultivars depended on the common (non fuscan) bacterial strain. Other study (Dursun et al. 2002) have found that bean cultivars and genotypes were different in their reaction to CBB pathogen as highly resistant, moderate resistant, resistant, tolerant and susceptible. The germplasm line XAN-159 developed from an interspecific cross between *P. vulgaris* and *P. acutifolius* (PI 319443) tested for CBB resistance at the International Center for Tropical Agriculture (CIAT) has been used in white and colored bean breeding programs in both the USA and Canada due to its high levels of CBB resistance. However, one cultivar XAN-310 (Dinkinesh) from this XAN line material evaluated in the current study showed moderately susceptible reaction. This fact indicates that genetic material resistant in one area can loss its resistant quality in another area either because the pathogenic variation in the pathogen pathosystem or because of the variation in the agronomic husbandry in the areas. Genetic studies on patterns of resistance to CBB in common bean indicated that it is polygenic resistance (Ferreira1 *et al*., 2003). Lack of geographical differentiation in the pathogen has important practical implication, as available host resistance genes are likely to be effective in controlling the disease in the diverse geographical areas (Mahuku *et al*., 2006).

In general, CBB ratings for the cultivars had similar reaction both under field and greenhouse experiments, but cultivars were resulted in less disease severity values when grown under field conditions especially at Melkassa. For example, as noted above, cultivar Ayenew was highly susceptible under green house trial while susceptible at both field trial sites and Mexcan-142 was also categorized as susceptible at Melkassa trial site whereas highly susceptible at Arsi Negele trial site and greenhouse evaluation. Similarly Awash Melka was showed moderate susceptibility reaction under Melkassa field trial but susceptible at Arsi Negele site and under greenhouse evaluation (Table 2). This variation is the variation due to weather condition which affect both the disease development and crop phonology. Tar'an *et al*. (2001) reported CBB resistance is associated with plant architecture, indeterminate growth habit and late maturity.

In the present study, cultivars also show significant difference in mean disease severity index, AUDPC and disease progress rate. The cultivars with susceptible disease reaction exhibit the highest percent severity index, AUDPC and disease progress rate; while cultivars with resistance disease reaction significantly reduce percent disease severity index, AUDPC and disease progress rate both under field and greenhouse trials. For instance, the highest disease severity index 80% at Arsi Negele, 71.48% at Melkassa and 82.4% in greenhouse trials on susceptible cultivar Mexcan-142, were significantly reduced to 18.89% on Gobe Rasha at Arsi Negele, 17.28% on GLP-2 at Melkassa and 28.4% on Gobe Rasha in greenhouse. Studies have shown that varietal resistance alone could reduce CBB epidemics (Mutlu *et al.*, 2005b). Tumsa (2007) in his varietal comparison study indicated that, CBB incidence, severity, AUDPC and disease progress rate was reduced in moderately susceptible Awash Melka as compared to the susceptible varieties Awash-1 and Mexican-142. Similarly, Belachew (2015) reported that resistant varieties, Awassa dumme and *AFR-702*, had reduced CBB development and increased seed yield.

Table 2. Mean disease severity response of bean cultivars against *Xanthomonas axonopodis* pv. *phaseoli*

Cultivar	Greenhouse experiment		Field experiment			
			Arsi Negele		Melkassa	
	Mean disease severity	Disease Reaction class	Mean disease severity	Disease reaction class	Mean disease severity	Disease reaction class
Acos Red	4.90	MS	4.80	MS	4.70	MS
Awash-1	6.57	S	6.83	S	6.30	S
Awash Melka	5.90	S	5.33	S	4.20	MS
Ayenew	7.20	HS	6.53	S	6.17	S
Batu	5.90	S	6.27	S	5.30	S
Chercher	3.80	MS	4.60	MS	3.97	MS
Chore	4.43	MS	4.40	MS	4.10	MS
Deme	3.67	MS	4.30	MS	3.57	MS
Dimitu	5.00	MS	4.77	MS	4.07	MS
Dinknesh	3.77	MS	4.80	MS	3.80	MS
ECAB0056	2.67	R	1.90	R	1.70	R
GLP-2	2.67	R	1.70	R	1.60	R
Gobe Rasha	2.57	R	1.97	R	1.77	R
Gofta	6.90	S	6.67	S	5.77	S
IBADO	4.57	MS	4.50	MS	4.10	MS
Melka Dima	4.67	MS	4.60	MS	4.07	MS
Melkie	4.89	MS	4.40	MS	4.17	MS
Mexcan-142	7.43	HS	7.20	HS	6.43	S
Nasir	4.57	MS	4.70	MS	4.07	MS
Roba	6.90	S	6.47	S	5.63	S
CV (%)	5.41		2.55		2.97	
LSD (0.05)	0.44		0.20		0.21	

The results of this investigation was in a conclusion that most bean genotypes commonly grown in the study area shows susceptible to moderately susceptible to *Xanthomonas axonopodis* pv. *phaseoli* (Xap) while genotypes GLP-2, ECAB0056 and Gobe Rasha were resistant. Therefore, those cultivars with CBB resistance reaction could be potential sources of CBB resistance which can be utilized in the breeding program to introgress resistance into susceptible commercial bean varieties that were preferred with other agronomic traits. Seed production and distribution of the identified resistant cultivars GLP-2, ECAB0056 and Gobe Rasha should be promoted as component of integrated CBB management strategy in order to sustain bean production and productivity. As it was reported by many scholars that Xap isolates from various regions of the world differed in their virulence on bean cultivars; if differences in pathotypes in Xap exist in Ethiopia it could complicate breeding for CBB

resistance and affect the durability of CBB-resistant cultivars. Therefore, further studies should be conducted using CBB pathotypes from different parts of the country to further determine the quality of resistance exhibited by the three resistant genotypes investigated in this study.

Table3: Percent Severity Index (PSI) of common bacterial blight on common bean cultivars at Arsi Negele

	PSI			
Cultivars	35DAP	49DAP	63DAP	77DAP
Acos Red	9.26d	20.37def	47.78d	53.33f
Awash-1	13.33bc	36.30ab	69.63a	75.93b
Awash Melka	16.3a	38.89a	46.67de	59.26e
Ayenew	14.44b	32.96bc	62.22b	72.59c
Batu	11.85c	31.85c	58.89c	69.63d
Chercher	5.18efg	20.37def	40h	51.11fgh
Chore	6.67e	20.74def	37.78i	48.89hi
Deme	2.59i	8.52j	39.63hi	47.78i
Dimitu	5.56ef	22.96d	43.7fg	52.96f
Dinknesh	3.33hi	9.63ij	42.22g	53.33f
ECAB0056	3.70ghi	12.59hi	15.93j	21.11jk
GLP-2	3.33hi	7.78j	15.1833j	18.89k
Gobe Rasha	2.96hi	10ij	16.67j	21.85j
Gofta	13.33bc	37.41a	63.33b	74.07bc
IBADO	4.07fghi	14.07gh	38.89hi	50.00ghi
Melka Dima	5.19efg	17.04fg	40.00h	51.11fgh
Melkie	4.44fgh	12.96hi	39.63hi	48.89hi
Mexcan-142	16.67a	39.63a	69.26a	80.00a
Nasir	4.44fgh	17.78efg	45.19ef	52.22fg
Roba	12.96bc	21.48de	62.22b	71.85cd
CV (%)	12.99	10.83	2.68	2.55
LSD (0.05)	1.71	3.88	1.98	2.26

Means labeled with the same letter in the same column are not significantly different. PSI = Percent Severity Index; DAP = days after planting

Table4: Percent Severity Index (PSI) of common bacterial blight on common bean cultivars at Melkassa

	PSI			
Cultivars	35DAP	49DAP	63DAP	77DAP
Acos Red	8.89de	24.07c	41.11e	52.22e
Awash-1	12.22b	31.48b	58.89ab	70.00ab
Awash Melka	11.11bc	34.07ab	35.56f	46.67f
Ayenew	7.78e	35.93ab	56.67b	68.52b
Batu	8.15e	23.70c	47.78d	58.89d
Chercher	3.7fg	11.48def	32.96gh	44.07gh
Chore	3.33fg	15.19d	34.81fg	45.56fg
Deme	4.07fg	8.52ef	28.52i	39.63i
Dimitu	7.41e	12.22def	33.70fg	45.19fg
Dinknesh	2.96fg	8.15ef	31.11h	42.22h
ECAB0056	2.96fg	10.00ef	13.33j	18.89j
GLP-2	3.70fg	12.59de	12.22j	17.78j
Gobe Rasha	2.96fg	7.41f	13.70j	19.63j
Gofta	10.00cd	37.04a	52.96c	64.07c
IBADO	2.59g	12.96de	34.44fg	45.56fg
Melka Dima	4.44f	15.19d	34.07fg	45.19fg
Melkie	4.44f	12.59de	33.70fg	46.30fg
Mexcan-142	14.44a	34.81ab	60.37a	71.48a
Nasir	4.07fg	12.96de	34.07fg	45.19fg
Roba	8.89de	15.93d	51.11c	62.59c
CV (%)	14.52	15.65	3.67	2.97
LSD (0.05)	1.54	4.87	2.25	2.33

Means labeled with the same letter in the same column are not significantly different. PSI = Percent Severity Index; DAP = days after planting

Table5: Percent Severity Index (PSI) of common bacterial blight on common bean cultivars under Greenhouse condition

	PSI		
Cultivars	35DAP	49DAP	63DAP
Acos Red	11.11fg	27.16f	54.32ef
Awash-1	24.69ab	41.97d	72.84c
Awash Melka	24.69ab	43.21cd	65.43d
Ayenew	25.93a	50.62ab	80.25ab
Batu	19.75c	34.57e	65.43d
Chercher	13.58def	28.40f	41.97h
Chore	13.58def	27.16f	49.38g
Deme	11.11fg	16.05jk	40.74h
Dimitu	14.81de	20.99ghi	55.56e
Dinknesh	11.11fg	17.28ijk	41.97h
ECAB0056	8.64gh	16.05jk	29.63i
GLP-2	7.41h	13.58k	29.63i
Gobe Rasha	8.64gh	14.81jk	28.40i
Gofta	22.22bc	53.09a	76.54bc
IBADO	11.11fg	22.22gh	50.62fg
Melka Dima	12.34ef	22.22gh	51.85efg
Melkie	12.34ef	18.52hij	53.09efg
Mexcan-142	24.69ab	46.91bc	82.72a
Nasir	11.11fg	24.69fg	50.62fg
Roba	16.05d	37.04e	76.54bc
CV (%)	13.88	8.91	5.29
LSD (0.05)	3.5	4.24	4.8

Means labeled with the same letter in the same column are not significantly different. PSI = Percent Severity Index; DAP = days after planting

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