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Occurrence and Importance of *Xanthomonas axonopodis* pv. *Phaseoli* in Common Bean (*Phaseolus vulgaris* L) Seed Produced under Different Seed Production System in Central Rift Valley of Ethiopia

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Abstract

Common bacterial blight of bean caused by the seed-borne bacteria *Xanthomonas axonopodis* pv. phaseoli (*Xap*) (Smith) *Vauterin* and *X. axonopodis* pv. *phaseoli* var. *fuscans* (Burkholder) Starr and Burkholder is one of the most constraint of common bean production all over the world. The pathogen is seed-borne and survives as long as the seed remains viable. Use of pathogen-free seeds has been the main method used to control the disease in most bean production areas, and detection of this pathogen in seeds is essential for effective disease control. This study was carried out to detect and characterize *Xap* in seed lots collected from different seed dealers and local markets in Central Rift Valley of Ethiopia. A semi-selective medium *Xanthomonas campestris* pv. *phaseoli* (XCP1) and yeast extract-dextrose-calcium carbonate agar (YDCA) were used to recover the bacterium from whole bean seed extract and direct seed plating respectively. The pathogenicity test was done on Mexcan-142 bean cultivar to confirm pathogen identification. Colonies of the bacterium were yellow, mucoid and convex on XCP1 media and zone of hydrolysis formed around them. Further biochemical test results also confirm that the colonies were gram negative, rod shape and hydrolyze starch, casein and Tween80. The results confirmed the presence of seed borne *Xap* in all seed dealers and local market seed lots in the study area. The result reveled *Xap* was prevalent in 79.27% of the total seed samples collected. Lower prevalence (21.43%), seed infection percentage (1.643%) and bacterial population were resulted in seed lots from Melkassa Agricultural Research Center seed lots; while the higher prevalence, seed infection percentage and bacterial population were observed in cooperative union, local market, and seed producer's cooperative seed lots. From the result, it can be concluded that *Xap* was potentially recovered from naturally infected seeds using XCP1 media and the pathogen is highly distributed in seed lots in the study area with high prevalence in farmer's produced seed lots. Therefore, seed plating on semi selective medium XCP1 can be used as standard method for routine analysis of *Xap* from bean seeds. Seed dealers in the study area should follow strict disease free seed production programs and farmers in the study area should be encouraged not to use local market and/or their own saved seeds for planting purpose.

Keywords: Common bacterial blight; Seed detection; *Xanthomonas axonopodis pv. Phaseoli*

Introduction

Common bacterial blight of common bean (*Phaseolus vulgaris* L) caused by the seed-borne bacteria *X. axonopodis* pv. *phaseoli (Xap)* (Smith) Vauterin and *X. axonopodis* pv. *phaseoli* var. *fuscans* (Burkholder) Starr and Burkholder is one of the most constraint of common bean production all over the world [1,2]. The disease causes both quantitative and qualitative yield losses and the yield loss reach up to 40%, depending on bean cultivar susceptibility and environmental conditions [3]. The pathogen distributed in most regions where common bean is cultivated except in arid tropical areas. It is a major disease in African countries such as Malawi [4], Uganda, Kenya, Burundi [5] and Tanzania [6]. It is also present in other south-eastern and southern Africa countries [7]. In Ethiopia, it is ranked among the most important diseases of common bean [8,9], and predominantly severe in areas characterized by high temperature, relative humidity and amount and intensity of rain fall [10].

Common bacterial blight disease is a seed-borne [11], and the pathogen survives as long as the seed remains viable [12]. Seed transmission is the primary means by which the pathogen is disseminated [13-15]. Internally and externally infested seeds are important sources of primary inocula for *Xap* [14,16]. Sutton and Wallen [17] reported that approximately one diseased seed in 10000 seeds are capable of causing an outbreak of blight. Weller and Saettler [15] also report that 1000 to 10000 bacteria per seed is the minimum needed to produce infected plants under field conditions. Therefore, the use of pathogen-free seeds has been the main method used to control the disease in most bean production areas [18-22] and detection of this pathogen in seeds is essential for effective disease control [23]. To limit this major inoculum source, specific seed production areas and seed certification were created in several countries [24]. These seed production areas should be located in areas where climate is considered to be non-conducive to diseases and/or where seed producers follow strict rules concerning the sanitary quality of stock seeds and cultural conditions like long rotations and isolated location of fields to limit the introduction and multiplication of inoculum.

However, in Ethiopia, most farmers retain bean seed for future planting and certified seed is seldom used even in the case of the new cultivars for which seed production has been organized with research centers, commercial seed production enterprise or farmer's seed production cooperatives. In most cases, certified seed is typically used in

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the first production year only. Then after, most farmers plant uncertified seed mainly saved from their own previous harvest, purchased from local markets or commercial seed dealers like farmers cooperative unions. Moreover, seeds from these commercial seed dealers were also uncertified for phytosanitary except cleaned by removing discolored and shriveled seeds during seed grading. However, symptomless, and slightly diseased seeds obtained from infected fields may rise to severely infected seedling. Therefore, there is a strong likelihood that such seed may act as sources of primary inoculum for seed borne diseases like common bacterial blight. This is particularly so for resource poor farmers who do not have access to certified seeds.

In line with the above-mentioned problems there was no strong work done and the pathogen status of bean seeds from different seed sources in the study area were not known. Therefore, this study was carried out to detect and characterize common bacterial blight pathogen from common bean seeds lots collected from different sources with specific objectives to evaluate their level of contamination with *Xap*.

Materials and Methods

Study area

This study was conducted in Central Rift Valley of Ethiopia during 2014 cropping season (Figure 1). The selection of Disticts/Woredas and Kebeles/Peasnt Assocition were made based on the potential of bean production and the presence of farmer's seed producer's cooperative with the intention to collect seed samples from different seed sources.

Seed sample collection

Common bean seeds were collected from various seed sources; which include seed producer's cooperative seed lots, farmer's cooperative unions, experimental site, and local markets. About 1 kg of seed samples were sampled from different parts of seed storage according to international seed health test (ISHT) sampling procedure. In seed producer's cooperatives and farmers cooperative unions seed lots, based on size of the seed lot one primary sample for each 500 kg were taken from each producer's seed lots. Markets in each locality where farmer's cooperative seed producers found were visited and about 1 kg of sample was collected randomly from 5 individuals in each locality. In case of experimental site about 1 kg of seeds from each cultivar was sampled. A total of 82 seed samples were collected from different seed sources in the study area. Each sample was collected separately in plastic bag and transported to Melkassa agricultural research center plant pathology laboratory and stored at +5°C in refrigerator till analysis. These samples were grouped in to four seed source as markets seeds, seed producers' cooperatives seeds, farmers' cooperative unions seed and research trial seeds.

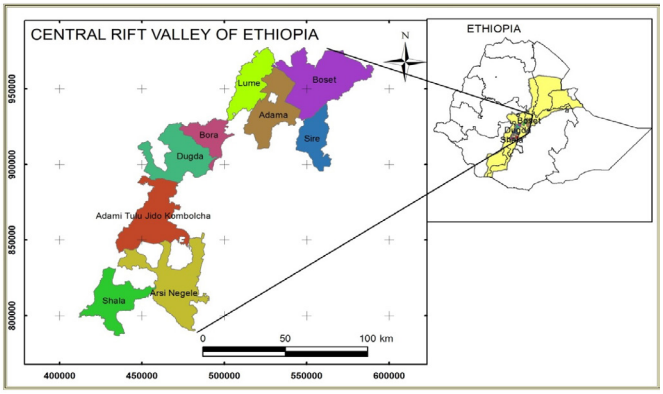


Figure 1: Central rift valley of Ethiopia.

Seed assay

Seed soak method: A working sample of 500 bean seeds was drawn from each sample and used for assay in line with International Seed Testing Association (ISTA) standards [25]. Seeds were soaked in seed extraction solution (0.85% saline with Tween20) in the proportion of 1:2 (1 g of seed in 2 ml of solution) and kept overnight at 5°C. After incubation, each suspension was thoroughly agitated and 10-fold dilution series (to 10^{-5}) of the seed extract was prepared. Afterwards, 0.1 ml of each undiluted and diluted extracts were spread on three plates of semi-selective media, *Xanthomonas campestris* pv. *phaseoli* (XCP1) as describe by International Seed Testing Association [26]. The plates were incubated at 28°C for five days. Then, the plates were visually assessed and all colonies typical of *Xanthomonas* genus (yellow pigment, convex, mucoid colony with inter margin) were examined and counted for each sample to determine number of colony forming units (cfu) per ml of seed extract. Suspected colonies of the pathogen were purified by sub culturing single colony on yeast extract-dextrose-calcium carbonate agar (YDCA). One colony of the purified suspect pathogen from each sample was selected and maintained on NA and YDCA slant at 4°C for further test.

Direct plating method : Similar to the seed soaking method, 500 randomly selected seeds from each seed lot were used for this assay. Sub samples of five of 100 seeds used as a replicate and 10 seeds plated per plate. Seeds were first sterilized by dipping in a 1% sodium hypochlorite solution for 30 seconds and rinsed in three changes of sterile distilled water (SDW) for three minutes to remove traces of sodium hypochlorite. The seeds were then placed on sterile filter paper to dry. Then seed were plated hilum downwards, on YDCA plate [27] and incubated at 28°C ± 2°C. After five days, the plated seeds were visually assessed for the presence of *X. axonopodis* pv. *phaseoli (Xap)* colonies under a stereomicroscope based on their morphological characteristics typical to *Xap*. The seeds from which *Xap* recovered were recorded as positive (+) and those from which *Xap* not recovered was negative (-). Mean percent seed infection level was calculated for each replicate from infection proportion per plate.

Pathogen characterization: Suspected colonies obtained from seed assay were subjected to a number of tests which included the gram reaction, casein hydrolysis, tween80 hydrolysis, starch hydrolysis and pathogenicity tests on bean plant.

Gram reaction: Grease on the slide was removed by flaming it several times. A small drop of water was placed on the middle of the slide. A small amount of the yellow pigment colonies was removed from the culture using a sterile wire loop and placed in the water drop. After mixing the cells with the water on the slide, the smear was dried by holding the slide over the flame. After cooling crystal violet was pipetted onto the surface and left for one minute. The stain was poured off the slide, washed with 70% alcohol, and iodine solution added for one minute before being rinsed off with water. Then safranin was applied and left for three minutes and then rinsed off with water before drying over the flame. The mounted specimen was examined under a compound microscope with 100x lens using oil immersion with no cover slip.

Casein hydrolysis test: Casein hydrolysis was demonstrated by streaking yellow-pigmented colonies on Skim milk agar medium. A single line streak inoculation was made from each isolate culture and plates were incubated at 28°C. After 48 h all incubated plate were observed for any clearing around the line of growth.

Starch hydrolysis test: The ability to degrade starch was performed by culturing the suspected isolate on starch agar media. A single streak inoculation of each isolate was made into the center of starch agar plates. The cultured plate was incubated at 28°C for 48 h in inverted position. After 48 h the surface of the plates were flooded with iodine solution and examined. A clear or yellow zone around a colony in otherwise blue media indicate a positive starch hydrolysis reaction.

Tween80 hydrolysis test: In Tween80 hydrolysis demonstration, a suspected colony of *Xap* isolate was streaked into the center of XCP1 plate. Plates were incubated at 28°C for 3-5 days. A milky zone around a colony growth indicates positive Tween80 hydrolysis.

Pathogenicity tests: For pathogenicity test four seeds of a susceptible bean variety; Mexcan-142 were planted in 20 cm diameter pots and after emergence two plants per pot maintained for inoculation. Selected isolates obtained from each sample in the seed assay experiments were cultured on nutrient agar NA, and then transferred onto nutrient broth NB and incubated on a shaker for 24 h at 25°C. Cells were suspended in distilled water and approximately adjusted to 10^8 CFU ml^{-1}. Plants were sprayed with water before inoculation to provide favorable conditions for infection. In addition, the floor of the greenhouse was covered with fiber sucks and kept wet to generate humidity in order to favor development of CBB. For inoculation, scissors contaminated with the bacterial suspension were used to cut the leaflets. Two leaflets of each plant were inoculated (always the middle leaflet). The plants were assessed for blight symptoms from seven days after inoculation.

Data analysis: Analysis of variance for colony populations/colony forming unit (cfu) and seed infection percentage were analyzed with SAS 9.2 computer software GLM procedure of nested design and mean separation test was performed by Duncan multiple range test.

Results

The seed assay Seed soak method: The seed extract plating result reveals that *Xap* was recovered from 65 samples of 82 total samples analyzed. This indicates that the pathogen is prevalent in 79.27% of the seed sample collected (Table 1). Low prevalence of Xap (21.43%) was recorded in seed samples collected from MARC trial sites seed lots while the highest prevalence was observed in seed samples collected from farmers' cooperative union seed lots. The result of *Xap* population confirmed that *Xap* colony populations were high for all positive samples ranging between 1.37×10^5 to 7.89×10^6 cfu/ml of seed extract. *Xap* colony population of MARC seed lots was significantly lower than the other seed sources (Table 2). There was also a significant difference in *Xap* population within markets and cooperative unions seed lots whereas there was no significant difference in *Xap* population within seed producer cooperatives and trial sites seed lots (Figure 2). Within the markets seed lots the lowest *Xap* population recovered from seed lots collected from Bofa local market, whereas the mean *Xap* populations of the other markets were statistically comparable.

Direct seed plating method: The result from direct seed plating assay reveals that *X. axonopodis* pv. *phaseoli (Xap)* was recovered from all sample positive in seed extract plating. The mean percentage of seed infection by Xap in seed samples significantly varied between seed sources (Table 3). The lowest seed infection percentage (1.643%) was recorded in MARC trial sites seed lots whereas the other seed sources had comparable seed infection level ranging from 5.575% to 9.156%. Within the seed sources, only markets seed lots were show significant difference in seed infection percentage. Low seed infection (2.44%) was observed in seed lots collected from Bofa local market, while the others market seed lots had comparable seed infection ranging from 6.08% to 10.60% (Figure 3).

Seed source	No of sample collected	No of *Xap* positive sample	% *Xap* prevalence
Markets	35	30	85.71
Seed producers' cooperatives	24	23	95.83
Farmers' cooperative unions	9	9	100.00
MARC trials	14	3	21.43
Total	82	65	79.27

Table 1: Occurrence of *Xap* in bean seed samples from different seed sources.

Seed source	CFU
Local markets	5.2375[a]
Seed producer cooperatives	5.7378[a]
Farmers' cooperative unions	6.5357[a]
MARC trial sites	1.2375[b]
CR	1.989 2.080 2.135
CV	1.0225
Means with the same letter are not significantly different; The figures in the table are log transformed mean bacterial population	

Table 2: Mean *Xap* population in bean seed sample from different seed sources.

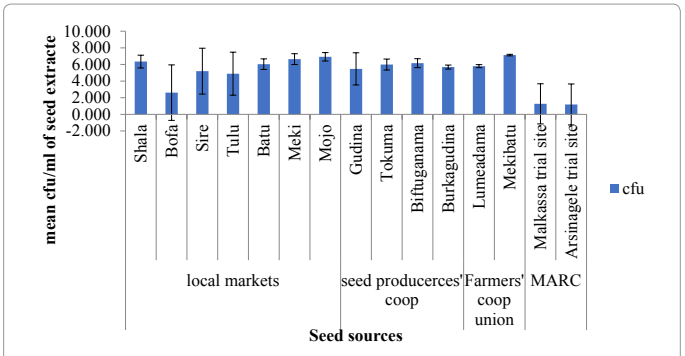


Figure 2: Mean log *Xap* population in seed lots within different seed sources.

Seed source	Infection%
Local markets	7.006[a]
Seed producer cooperatives	5.575[a]
Farmers' cooperative union	9.156[a]
MARC	1.643[b]
CR	3.724 3.895 3.997
CV	18.27
Means with the same letter were not significantly different	

Table 3: *Xap* infection level of bean seed in different seed sources.

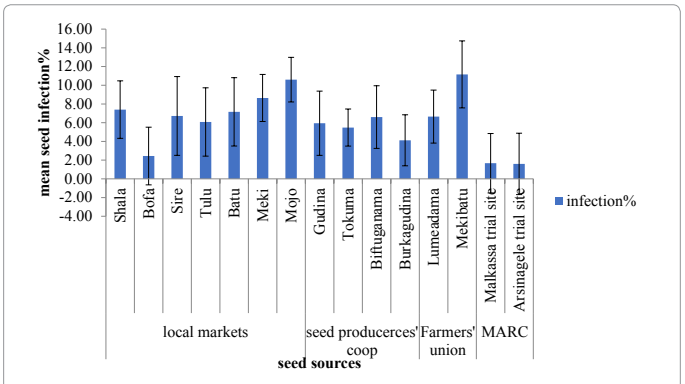
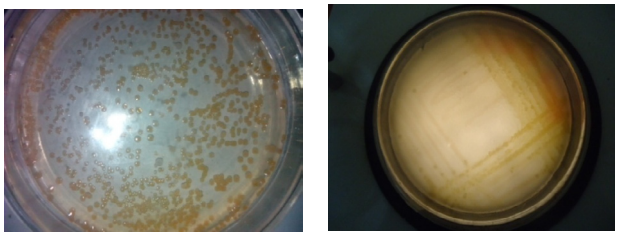


Figure 3: Mean bean seed infection in seed lots within seed sources.



(a) (b)

Plate 1: *X. axonopodis* Pv. *phaseoli* colonies on XCP1 (a) and YDCA (b) media.

Characteristics	*Xap* reaction
Gram reaction	-
Casein hydrolysis	+
Tween80 hydrolysis	+
Starch hydrolysis	+
Pathogenicity on bean	+
Key (+): *Positive* identification (-): Negative identification	

Table 4: Biochemical characteristics of *Xap*.

Pathogen characterization: The bacterial isolates recovered from seed samples were classified as Xanthomonas like, based on yellow pigment, convex mucoid morphology (Plate 1a and 1b).

The colonies of *Xap* on XCP1 medium appeared as bright yellow, mucoid, convex, smooth, round with entire margins and surrounded by zone of starch and tween80 hydrolysis. The biochemical test results also confirm that the colonies were gram negative, rod shape and hydrolyze starch, cuisine and Tween80. The observed characteristics of *Xap* were summarized in Table 4. Other unidentified bacteria with creamy white, flat circular colony were also recovered from the seed at the same time.

In the pathogenicity test on bean plant, all suspected isolate recovered from seed assay induced symptoms ten days after inoculation. The symptom was first appeared around growing tips as small water-soaked spots on the underside leaves. The lesions gradually enlarge and join to develop into large irregular shape lesions. Then the lesions become dry brown and surrounded by a narrow yellow border.

Discussion

Seed assays are the important steps in the seed system and the most reliable methods of determining whether or not seeds are infected with seed borne pathogens [28]. Although several techniques like serology [29,30] and polymerase chain reaction [31,32] have been developed for detection of CBB pathogen in seed, isolation on semis elective media remains the most widely used detection method [33-35]. In the present study, the morphological characteristics of the bacterium on the XCP1 medium, biochemical and pathogenicity test results confirmed that the pathogen recovered from seed was *X. axonopodis* pv. *phaseoli (Xap)* and this result was in agreement with the finding of [33]. In this study the bacterium, *Xap* was successfully isolated by plating the extract obtained from the whole seeds onto the semi-selective medium XCP1. This indicated the suitability of XCP1 for detection of *Xap* from seed lots. Remeeus and Sheppard [33] report that ISTA/ISHI evaluate several semi-selective media for use in the detection of *Xap* in bean seed including BBD, MT, YSSM, MXP and PTSA media and find that no significant difference was observed in the detection of *Xap* between media. However, characteristics of some media proved more suitable for routine testing of bean seed than others and XCP1 was consistently found to have good selection for both fuscans and non-fuscans types and was preferred by participants for ease of detection.

The current study also revealed that *Xap* was prevalent in all kinds of seed system in the study area including seed from research center seed multiplication site. The average prevalence was 79.27% and it varies from seed source to seed source and within the seed sources. This variation was probably because different seed source follow different seed production management including cultural practices during seed production and handling. This result was in agreement with the finding of Kedir et al. [36] where they reported that *Xap* is associated with 59.1% and 75.0% of bean seed sample produced respectively in intercropping and sole cropping system in Eastern Ethiopia.

Results of seed infection level and bacterial population of the seed lots in this study also showed that there was heavy infection of *Xap* in bean seed lots in the study area. The lowest seed infection level (1.643%) was found in seed lots from Melkassa Agricultural Research Center (MARC) while the other seed sources had higher seed infection level ranging from 5.575% to 9.156% with heavy bacterial population. Karavina et al. [37] report seed borne *X. phaseoli* is common in both retained and certified common bean seed lots in Zimbabwe with the former having significantly higher bacterial population levels. In Canada, 0.5% of seed infection level has led to disease epidemics [38]. Weller and Saettler [15] reported that the minimum population of *Xap* to initiate common bacterial blight infection is 10^3 to 10^4 which, shows bean seed from all seed sources in current study was heavily infected by *Xap* and capable to cause CBB epidemic when there is favorable environmental conditions. Moreover, the result shows that the seed samples from farmer's seed lots (seed collected from local market, seed producer cooperatives and cooperative unions) had higher levels of pathogen infection compared to seed from trial site. This might be because of seed production management system the seed dealers' follow including site selection, field sanitation and other disease management practices and seed grading problems. During the study, it was observed that all seed dealers in the study area have less seed grading practices and they only remove sherveled and seeds with visible disease symptom. However, contamination of seeds without symptom expression during the growing season represents a risk for eventual disease outbreaks [39].

Conclusion

The experiments confirmed the presence of *Xap* as a seed borne pathogen in all different seed sources in the study area. This wide spread distribution of *Xap* in all seed production system and the fact that CBB pathogen build up over time which may result in high risk of disease outbreak indicate that, strict seed phytosanitary certification and disease free seed production program as a primary *Xap* management in the study are in particular and as a country in general should be given apriority.

Farmer's seeds (seed collected from local market, seed producer cooperatives and cooperative unions) had higher levels of pathogen infection compared to seed from trial site; therefore, seed dealers should have to get expertise/extension services and farmers in the area should have to be encouraged not to use uncertified seeds for planting purpose. Moreover, as seed inoculum is the primary source of infection seed dealers in the study area should have to follow exclusion disease management strategies like producing seeds in off season and in dry cooler areas so that they able to supply disease free seeds. A specific standard method for detection of *Xap* from bean seeds has not reported in Ethiopia so far, and the result shows that the method used in this study was effective and suitable in isolating this bacterium from naturally infected bean seeds. Therefore, seed plating on semi selective medium XCP1 can be used as standard method for routine analysis of *Xap* from bean seeds.

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References

1. Mabagala RB, Saettler AW (1992) An improved semi-selective media for the recovery of Xanthomonas *campestris* pv. phaseoli. Plant Dis 76: 443-446.
2. Opio AF, Allen DJ, Teri JM (1996) Pathogenic variation in *Xanthomonas campestris* pv. phaseoli, the causal agent of common bacterial blight in Phaseolus Beans. Plant Pathol 45: 1126-1133.
3. Mutlu N, Miklas PN, Steadman JR, Vidaver AV, Lindgren D, et al. (2005) Registration of pinto bean germplasm line ABCP-8 with resistance to common bacterial blight. Crop Sci 45: 806-807.
4. Edje OT, Mughogho LK, Rao VP, Msuku WAB (1981) Bean production in Malawi. In: Amaya S, Motta FM, (Eds). Regional Workshop on Potential of Field Beans in Eastern Africa, Proceedings, Cali, Colombia, Centro Internacional de Agricultura Tropical, East Africa.
5. Opio AF, Teri JM, Allen DJ (1993) Studies on seed transmission of *Xanthomonas campestris* pv. phaseoli in common beans in Uganda. Afr Crop Sci J 1: 59-67.
6. Karel AK, Ndunguru BJ, Price M, Semuguruka SH, Singh BB (1981) Bean production in Tanzania. In: Amaya S, Motta FM (Eds). Regional Workshop on Potential for Field Beans in Eastern Africa, Proceedings, Cali, Colombia, Centro Internacional de Agricultura Tropical, East Africa.
7. EPPO (European and Mediterranean Plant Protection Organization) (1997) Data Sheets on Quarantine Pests: *Xanthomonas axonopodis* pv. phaseoli. Prepared by CABI and EPPO for U.
8. Habtu A, Sache I, Zadoks JC (1996) A survey of cropping practices and foliar diseases of common bean in Ethiopia. Crop Prot 15: 179-186.
9. Fininsa C (2003) Relationship between common bacterial blight severity and common bean yield loss in pure stand and maize common bean intercropping. Int J Pest Manag 49: 177-185.
10. Habtu A, Bosch F, Zadoks J C (1995) Focus expansion of common bean rust in cultivar mixture. Plant Pathol 44: 503-509.
11. Schaad NW (1982) Detection of seed borne bacterial plant pathogens. Plant Dis 66: 885-890.
12. Hirano SS, Upper CD (1983) Ecology and epidemiology of bacterial pathogens. Ann Revi Phytopathol 21: 243-269.
13. Cafati CR, Saettler AW (1980) Transmission of *Xanthomonas phaseoli* in seeds of resistant and susceptible Phaseolus genotypes. Phytopathol 70: 638-640.
14. Weller DM, Saettler AW (1980a) Colonization and distribution of *Xanthomonas phaseoli* in field-grown navy beans. Phytopathol 70: 500-506.
15. Weller DM, Seattler AW (1980b) Evaluation of seed borne *Xanthomonas phaseoli* and *Xanthomonas phaseoli* var fuscans as primary inoculum in bean blights. Phytopathol 70: 148-152.
16. Hall R (1994) Compendium of bean diseases. The American Phytopathological Society, New York, USA.
17. Sutton MD, Wallen VR (1970) Epidemiological and ecological relations of *Xanthomonas phaseoli* and *Xanthomonas phaseoli* var. fuscans on beans in south western Ontario, 1961-1968. Can J Bot 48: 1329-1334.
18. Yoshii K (1980) Common and fuscous blights. Pages 155-172 in: bean Production Problems. Schwartz, H.F. and Galvez GE (Eds.) Center of International Agriculture Tropic (CIAT). California. Colombia, 424 pp.
19. Saettler AW, Cafati R, Weller DM (1986) Non-over wintering of *Xanthomonas bean* blight bacteria in Michigan. Plant Dis 70: 285-287.
20. Abo-Elyousr KAM (2006) Induction of systemic acquired resistance against common blight of bean (Phaseolus vulgaris) caused by Xanthomonas campestris pv. phaseoli. Egypt J Phytopathol 34: 41-50.
21. Berova M, Stoeva N, Zlatev Z, Stoilova T, Chavdarov P (2007) Physiological change in bean (*Phaseolus vulgaris* L.) leaves infected by the most important bean disease. J Cent Eur Agric 8: 57-62.
22. Darrasse A, Bureau C, Samson R, Morris C, Jacques MA (2007) Contamination of bean seeds by *Xanthomonas axonopodis* pv. phaseoli associated with low bacterial densities in the phyllosphere under field and greenhouse conditions. Eur J Plant Pathol 119: 203-215.
23. Lahman LK, Schaad NW (1985) Evaluation of the “Dome Test” as a reliable assey for seedborne bacterial blight pathogens of bean. Plant Dis 69: 680-683.
24. Webster DM, Temple SR, Galvez GE (1983) Expression of resistance to *Xanthomonas campestris* pv. phaseoli in *Phaseolus vulgaris* under tropical conditions. Plant Dis 67: 394-396.
25. Draper SR (1995) International Rules for Seed Testing. Seed Science and Technology 3(ii) 331.
26. Grimault V, Olivier V, Rolland M, Darrasse A, Jacques MA (2014) Seed health testing methods. 7-021: Detection of Xanthomonas axonopodis pv. phaseoli on Phaseolus vulgaris. In: ISTA International rules for seed testing. Annexe to Chapter 7: Seed health methods 7-021. International Seed Testing Association, Basserdorf, Switzerland, 1-20.
27. Schaad NW (1988) Laboratory Guide for Identification of plant pathogenic bacteria. (2ndedn), The American Phytopathological Society, Minnesota, USA.
28. Rideout MS, Roberts SJ (1997) Improving quality control procedures for seed borne pathogens by testing sub-samples of seeds. Seed Sci Technol 25: 195-202.
29. Malin EM, Roth DA, Belden EL (1983) Indirect immunofluorescent staining for detection and identification of *Xanthomonas campestris* pv. phaseoli in naturally infected bean seed. Plant Dis 67: 645-647.
30. Sheppard JW, Roth DA, Saettler AW (1989) Detection of *Xanthomonas campestris* pv. phaseoli in Bean. In: Saettler AW, Schaad NW, Roth DA (Eds.) Detection of bacteria in seed and other planting material. The American Phytopathological Society, Minnesota, USA. 17-29 p.
31. Audy P, Braat CE, Saindon G, Huang HC, Laroche A (1996) A rapid and sensitive PCR-based assay for concurrent detection of bacteria causing common and halo blights in bean seed. Phytopathol 86: 361-366.
32. Molouba F, Guimier C, Berthier C, Guenard M, Olivier V, et al. (2001) Detection of bean seed-borne pathogens by PCR. Acta Horticult 546: 603-607.
33. Schaad NW (1982) Detection of seedborne bacterial plant pathogens. Plant Dis 66: 885-890.
34. Sheppard JW, Kurowski C, Remeeus PM (2007) International Rules for Seed Testing, 7-021: Detection of *Xanthomonas axonopodis* pv. phaseoli and *Xanthomonas axonopodis* pv. phaseoli var. fuscans on Phaseolus vulgaris. Bassersdorf, Switzerland: International Seed Testing Association (ISTA).
35. Balaz J, Popovi T, Vasi M, Nikoli Z (2008) Elaboration method for the detection of Xanthomonas axonopodis pv. phaseoli in bean seeds. Pestic Fitomed 23: 89-98.
36. Kedir O, Setegn G, Kindie T (2014) Assessment of common bean (*Phaseolus vulgaris* l.) seed quality produced under different cropping systems by Smallholder farmers in eastern Ethiopia. Afr J Food Agric Nutr Dev 14: 8566.
37. Karavina C, Chihiya J, Tigere TA (2008) Detection and characterization of *Xanthomonas phaseoli* (e. f. sm) in common bean (*Phaseolus vulgaris* L) seeds collected in Zimbabwe. J Sustain Dev 10: 105-119.
38. Zaumeyer WJ, Thomas HE (1957) A monographic study of bean diseases and methods for their control. US Department of Agriculture Technical Bulletin, 868, 255 pp.
39. Grum M, Camloh M, Rudolph K, Ravnikar M (1998) Elimination of bean seed-borne bacteria by thermotherapy and meristem culture. Plant Cell Tissue Organ Cult 52: 79-82.