

# Reaction of Cowpea Genotypes to Bacterial Blight (*Xanthomonas campestris* sp. *Vignicola*) Disease in Ghana

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**Abstract** The productivity of cowpea is constrained by a variety of biotic and abiotic factors. Insects, fungi, bacteria, parasitic plants and nematodes are the major biotic stresses, and drought, salinity and heat are among the major environmental limitations to cowpea productivity. Among the diseases, bacterial blight caused by *Xanthomonas axonopodis* sp. *Vignicola* (Burkholder) Dye is one of the diseases posing a serious challenge to cowpea production in Ghana and the rest of Africa where the crop is usually cultivated. The objective of the study was to identify Cowpea genotype that are resistant to bacterial blight. Thirty-one cowpea lines collected from research institutions (SARI-Nyankpala, PGRRI-Bunso, IITA-Kano-Nigeria and CRI-Kumasi) composed of landraces and released varieties were used for the trial. These were grown in polybags of 20 plants per accession in a Completely Randomize Design with four replications. The plants were inoculated with *X. vignicola* cultures when the plants were three weeks old. Results indicate that 64.5% of the plant total was moderately resistant, 22.6% were resistant and 12.9% were susceptible. The genotypes GH4025 and GH2347 were found to be promising resistant genotypes. The most pathogenic of all the strains was Ohawu 1 followed by Nyankpala 1 and CRI 2 respectively. Thermos resistant genotypes GH4025, GH4327 and IT97K-1069-6 exhibited higher level of resistance in all the three strains and the genotypes TVu7778, GH7889, IT84S-2246-4 and GH7225 exhibited similar levels of susceptibility to the three strains of the bacterial blight isolates. However, bacterial blight symptoms were observed on leaves inoculated with  $10^8$  cfu g<sup>-1</sup> bacterial suspensions.

**Keywords:** cowpea, disease, abiotic factors, biotic factors, inoculation

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## 1. Introduction

Cowpea [*Vigna unguiculata* (L.) Walp, Fabaceae (2n = 22)] is an important food legume and an essential component of cropping systems in the drier regions of the tropics [1]. At least 12.5 million hectares of cowpea are cultivated with annual production of over 3 million metric tons worldwide [1]. The largest production is in Africa, with Nigeria and Niger predominating, while Brazil, Haiti, India, Myanmar, Sri Lanka, Australia, the U.S., Bosnia, and Herzegovina all have significant production. Worldwide area of production of cowpeas is approximately 10.1 million hectares. More than 5.4 million tons of dried cowpeas are produced worldwide, with Africa producing nearly 5.2 million. Nigeria, the largest producer, and

consumer accounts for 61% of production in Africa and 58% worldwide. As many as a thirds of the world's people do not meet their physical and intellectual potential because of vitamin and mineral deficiencies [2]. In Africa, it is estimated that over 200 million people consume cowpea daily [3]. Cowpea is consumed in many forms: the young leaves, green pods, and green seeds are used as vegetables; dry seeds are used in various food preparations, and the hulls are fed to livestock as a nutritious supplement to cereal fodder. In West and Central Africa, cowpea is of major importance to the livelihoods of millions of people providing nourishment and an opportunity to generate income. Trading fresh produce and processed food and snacks provide rural and urban women with the opportunity for earning cash income and, as a major source of protein, minerals, and vitamins in daily diets. Also, it positively impacts on the health of

women and children. The bulk of the diet of rural and urban poor Africa consists of starchy food made from cassava, yam, plantain and banana, millet, sorghum, and maize. The addition of an even small amount of cowpea ensures the nutritional balance of the diet and enhances the protein quality by the synergistic effect of high protein and high lysine from cowpea and high methionine and high energy from the cereals [4]. In spite of the great importance of this crop to the people where it is grown, its productivity is constrained by a variety of biotic and abiotic factors. Insects, fungi, bacteria, parasitic plants, and nematodes are the major biotic stresses, and drought, salinity, and heat are among the major environmental limitations to cowpea productivity [5,6]. Besides fungal and viral diseases, cowpea bacterial blight (CoBB) and pustules caused by *Xanthomonas axonopodis* sp. *vignicola* (Xav) [7] formerly *X. campestris* sp. *vignicola* [8] is the most important disease of cowpea. CoBB is prevalent in all major cowpea growing areas of the world [9], [10] causing severe grain yield loss of more than 64% in some areas of West Africa [11]. When highly susceptible cultivars are sown the crop may even be completely destroyed [12]. The symptoms of CoBB appear as tiny, water-soaked, translucent spots, which are more clearly visible from the abaxial surface of the leaves [13]. The spots enlarge, coalesce and develop to big necrotic spots, usually with a yellow halo, leading to premature leaf drop. The pathogen also invades the stem causing cracking with brown stripes. Pod infection appears as dark green water-soaked areas, from where the pathogen enters the seeds and causes discoloration and shrivelling [11]. Cowpea bacterial blight (CoBB) is seed-borne [11] and the pathogen can be spread by wind-driven rain and insects [14] but also crop debris and weeds can play a role as inoculum sources [15].

Bacterial blight caused by *Xanthomonas axonopodis* sp. *vignicola* is an important and widespread disease of cowpea (*Vigna unguiculata* L. Walp) in many tropical and subtropical countries [16-21]. The pathogen is seed-borne and seed transmitted Shekhawat & Patel, (1977) and causes discoloration of seeds and cotyledons, seedling mortality, stem cankers, bushy and stunted growth, leaf and pod blight [18]. The resultant attack causes a reduction in yield and lowers the quality of seeds [19,22,23]. Losses range from 2.66 to 92.24% according to cultivar and stage of infection [17]. One of the major goals of cowpea breeding and improvement programs is to combine resistance to numerous pests and diseases and other desirable agronomic traits, such as those governing maturity, photoperiod sensitivity, plant type, and seed quality [18]. There is, therefore, the need for progressive work done to eradicate or reduce the effect of this problem. Therefore the aim of the study was to investigate Cowpea genotype that are resistant to bacterial blight.

## 2. Materials and Method

The evaluation of variation in the reaction of agronomic characters of cowpea under inoculation with *Xanthomonas campestris* sp. *vignicola* was carried out at the lathhouse at Cocoa Research Institute of Ghana, Akim Tafo in the Eastern Region of Ghana.

### 2.1. Source and Isolation of Pathogen

Cultures of *X. campestris* sp. *vignicola* were isolated from bacterial blight infected leaves of cowpea collected from the Savanna Agricultural Research Institute (SARI - Nyankpala), Plant Genetic Resource Research Institute (PGRRI - Bunso), Ohawu Agricultural College (Volta Region) (Plate 1), Crop Research Institute of Ghana (CRI – Fumesua, Kumasi) (Plate 2 and Plate3), and Sunyani in Brong Ahafo region. A laboratory guide for identification of plant pathogenic bacteria designed by [24], was used with slight modification for the isolation. The infected leaves were first surface sterilized with 70% ethanol. Two grams of each infected leaf was weighed and transferred into sterilized mortar and pestle. Each infected leaf sample was ground in 18 mL of maximum recovery diluent (MRD) (Peptic digest of animal tissue 1.0g, sodium chloride 8.5 g, pH 7.0±0.2 at 25°C) to prepare pathogen concentration of each sample. A nutrient broth (Lab-Lemco powder 1.0g, Yeast extract 2.0g, Peptone 5.0 g, sodium chloride 5.0 g, pH 7.4 ± 0.2) solution was used to perform serial dilution of concentrations from 10<sup>-1</sup> to 10<sup>-5</sup> where 1ml of each stock was drawn and transferred into 9 ml of nutrient broth solution. This was vortexed to obtain a uniform mixture. The uniform mixture obtained at each stage served as the stock for the next dilution till 10<sup>-5</sup> for each pathogen isolate. One hundred microliters (100 µL) of each pathogen concentration was spread on a nutrient agar medium (bact. peptone 5 g, beef extract 3g, NaCl 5 g, agar 18 g, distilled water 1 mL, pH 6.8 - 7.8) at 30 ± 1°C for 48 h) prepared and set in Petri dishes. The preparation of the nutrient agar medium was done in a laminar flow hood at the microbiology laboratory of Cocoa Research Institute of Ghana. The nutrient agar media were incubated in an incubator at 30°C for 24 hrs. After 24 hrs there was growth on all the nutrient agar media with different pathogen concentration levels. Each Petri dish produced colonies which were yellow and pale yellow in colour. A colony counter machine was used to count the number of colony growth of each pathogen concentration on the Petri dish which varied according to the pathogen concentration. Pure colonies were produced through subsequent sub-culturing by streaking on nutrient agar medium at 30±1°C for 48 hrs. Cultures of the isolates were maintained on nutrient agar slants and sub-cultures were preserved by transferring the 24 hrs matured pathogen into 50% glycerol solution and stored at 4 – 6°C. The pathogens from pure cultures were transferred into a nutrient broth (NB) solution and kept overnight in an incubator for multiplication. The concentrations of the solutions were determined using UV/visible spectrometer. Different pathogen concentrations of the selected isolates were used to inoculate healthy plants with a hand-operated atomizer.



Plate 1. Ohawu 1 from Ohawu Agricultural College (Akatsi District)



**Plate 2.** CRI 1; from Crop Research Institute (Fumesua-Kumasi)



**Plate 3.** CRI 2; from Crop Research Institute (Fumesua – Kumasi)

## 2.2. Identification of the Bacterial Colonies

The pathogens isolated from the infected leaf samples were subjected to various biochemical tests such as gram reaction test, nutrient agar + 5% glucose test, nitrate reduction test, citrate utilization test and salt tolerant test including a pathogenicity test with the accessions including a known variety from IITA (Tvu 7778 susceptible to bacterial blight). The 24 hrs old isolates from the various locations were used to inoculate the first trifoliate leaves of three weeks old cowpea plants grown for the preliminary studies by inoculating the bacteria isolates with a hand-operated atomizer. The plants to be inoculated were well watered 4 hrs prior to inoculation. Five plants were inoculated with each pathogenic isolate at the concentration of 0.05 optical density equivalent to  $10^7$  c.f.u  $g^{-1}$ . The first two trifoliate leaves were slightly injured at the abaxial with a sharp toothpick to help with the establishment of the pathogens.

The symptoms of the bacterial blight disease were scored as follows;

- 0 = no symptoms
- 1 = (only leaf spot symptoms visible, i.e. translucent and water-soaked spots),
- 2= (leaf blight: 10–50 % leaf area infected, inoculated trifoliate intact),
- 3= (severe blight symptoms: (more than 50% leaf area infected, inoculated trifoliate intact),
- 4= (inoculated trifoliate is shed).

The plants began exhibiting the symptoms of the bacterial blight 28 days after inoculation. Based on the scores the virulence pathogenic isolates, Ohawu 1 (yellow colony from Ohawu), CRI 2 (pale yellow colony from Crop Research Institute), Sunyani, Akim Tafo 1 (yellow colony from Akim Tafo), Nyankpala 1 (yellow colony from Nyankpala) and Bunso 1 (yellow colony from Bunso)

exhibited various levels of the symptoms of the bacterial blight disease. The pathogens of the infected leaves at the end of the pathogenicity test were re-isolated from the leaves and taken through the various biochemical tests as mentioned earlier to further confirm their level of pathogenicity. This reduced the prospective bacterial blight pathogens to three, Ohawu 1, Nyankpala 1 and CRI 2.

## 3. Gram Reaction Test

The Gram-reaction of each isolate was determined following the staining procedure as described by [25]. A drop of 3% KOH was placed on a microscope slide using a Pasteur pipette. A cooled sterilized loop was used to transfer part of a single colony from the agar medium onto the microscope slide and mixed with another microscope slide. The samples that dissolved in the 3% KOH solution to form suspension were classified as Gram-Positive and those that formed mucoid or slimy substance were classified as Gram-Negative.

### 3.1. Growth on Nutrient Agar + 5 % Glucose

Each isolate was streaked on nutrient agar with 5% glucose (Nutrient agar, 11.5g; 5% glucose in 500 mL of distilled water with pH 7 and autoclaved at 121°C for 15 minutes) and incubated at 28°C for 48-72 hours [25].

#### 3.1.1. Nitrate Reduction Test

The ability of the isolates to reduce nitrate to nitrite was evaluated in a test medium that contains  $KNO_3$  0.5g; Peptone, 2.5g; Yeast extract, 1.5g and Agar, 1.5g in 500mL of distilled water, sterilized at 121°C for 15 minutes in tubes. Each isolate was inoculated by stabbing and sealed with 3 mL sterilized molten agar to avoid false positives and inoculated at 28°C.

#### 3.1.2. Citrate Utilization Test

Citrate utilization of the isolates was tested using Simon's citrate agar slants ( $NH_4H_2PO_4$  1g;  $K_2HPO_4$  1g; NaCl, 5g;  $MgSO_4 \cdot 7H_2O$ , 0.2g; sodium citrate, 2g; agar, 15g; bromothymol blue, 0.08g in 1 litre distilled water (pH 6.9) and autoclaved at 121°C. for 15 minutes). A loopful from each isolate was streaked on the slant and incubated at 28°C. for 48-72 hours. A change of colour from green to blue was taken as positive for citrate utilization and uninoculated tubes were used as negative control [26].

#### 3.1.3. Salt Tolerance Test

Isolates were inoculated into nutrient broth with 0%, 1%, 2%, 3%, 4% and 5% NaCl concentration to evaluate their salt tolerance. Inoculated salt-free (0%) nutrient broth was used as positive control and uninoculated broth of each salt concentration was used as negative control and the presence or absence of growth was recorded [26].

## 4. Statistical Analysis

The data collected from visual scoring of the disease was first subjected to a normality test using Minitab:



version 13. After confirming the normality of the data it was then subjected to mean separation using Mstat.

Foliar disease severity data of genotypes were subjected to analysis of variance and Duncan Multiple Rank Test (test level 5%). This was used to determine significant differences between the reactions of the genotypes to the bacterial blight isolates

#### 4.1. Screening of the Assembled Lines

The pure cultures of each of the three prospective bacterial blight pathogens (Ohawu 1, Nyankpala 1 and CRI 2) were multiplied separately in 1 liter of nutrient broth (NB) solution overnight (12-24hrs). The bacterial blight concentration was adjusted on UV/visible spectrophotometer at 420 nm to  $10^8$  colony forming unit  $g^{-1}$ , at 0.5 optical density. The solutions were then diluted by adding and mixing gently a sterilized NB solution to  $10^7$  c.f.u  $g^{-1}$  equivalent to 0.05 optical density and used to inoculate both the abaxial and adaxial parts of the first two healthy trifoliolate leaves by spraying the inoculum with a hand-operated atomizer. The stalks of the plants were also inoculated by dipping the tip of the needle into the inoculum and slightly pierced through the stalk. The plants to be inoculated were thoroughly watered four hours prior to the inoculation. The plants to be sprayed were slightly injured at the adaxial part by using sharp toothpicks. The inoculation was done by spraying the inoculum with a hand-operated atomizer on both the adaxial and abaxial surfaces of the first two trifoliolate leaves until water-soaked spots appeared as described by [11,27]. The known resistant (IT00K-1263, IT99K-111-1) and susceptible (IT84S-2246-4, TVU-7778) varieties from IITA were included in the experiment as controls. The control varieties were also inoculated with distilled water. The inoculated plants were covered with transparent polythene bags.

#### 4.2. Screening of Germplasm for Reaction to Bacterial Blight

Thirty-one cowpea lines collected from research institutions (SARI-Nyankpala, PGRRI-Bunso, IITA-Kano-Nigeria, and CRI-Kumasi) were screened. The inoculation was done when the plants were three weeks old. The adaxial part of the leaves was injured with toothpicks to create an entrance for the establishment of the pathogen. This was carefully done not to destroy the leaves. The second and third trifoliolate leaves of each plant were held against the

palm and inoculated by spraying the bacterial blight inoculum on both the abaxial and adaxial surfaces with the most pathogenic isolate (Ohawu 1) from a distance of about 2 cm using a hand-operated atomizer. The inoculated plants were covered with clear poly bags to conserve moisture to enhance the establishment of infection. The plants were rated for incidence and severity 28 days after inoculation and subsequently at 7 days intervals for 4 weeks using a scale of 0 (no symptom) to 4 (inoculated trifoliolate leaves shed).

### 5. Results and Discussion

#### 5.1. Biochemical Tests

The isolate from various locations were subjected to biochemical tests to categorize them.

#### 5.2. Gram Reaction Test

From Table 1, Tafo isolates 1 and 2, Bonsu isolate and Ohawu isolate 2 were shown to be Gram-Positive bacteria. Ohawu 1, Sunyani, Nyankpala 1 and 2 and CRI 1 and 2 isolates also were Gram-Negative bacteria.

#### 5.3. Growth on Nutrient Agar + 5 % Glucose

Mucoid and yellow colony were recorded for Tafo 1, Bonsu, Ohawu 1, Sunyani, Nyankpala 1 and CRI 1 isolates (Table 2). The pale yellow samples also remained the same colour and these isolates were Tafo 2, Ohawu 2, Nyankpala 2 and CRI 2.

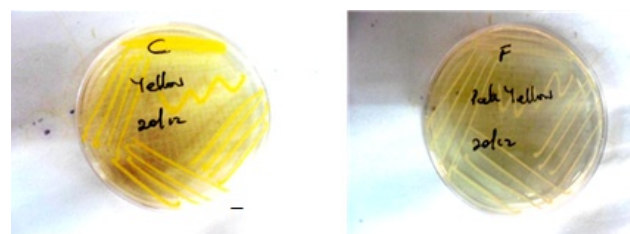


Plate 4. Bacterial blight isolates; yellow colour (A) and pale yellow (B)

#### 5.4. Nitrate Reduction Test

Observation was made after 3, 5 and 7 days of inoculation. Bubble formation beneath the upper agar layer was observed only in F2 as shown in Table 2.

Table 1. Reaction of isolates to different biochemical tests

Test Isolates	Colony Color	Gram Reaction Test	Growth on NA + 5% Glu	Nitrate Reduction	Citrate Utilization	Salt Tolerance
Tafo1	Yellow	+	+	-	+	+
Tafo 2	Pale Yellow	+	+	-	+	+
Bonsu	Yellow	+	+	-	-	+
Ohawu1	Yellow	-	+	-	+	+
Ohawu2	Pale Yellow	+	+	-	-	+
Sunyani	Yellow	-	+	-	+	+
Nyankpala 1	Yellow	-	+	-	+	+
Nyankpala2	Pale Yellow	-	+	-	+	+
CRI 1	Yellow	-	+	-	-	+
CRI 2	Pale Yellow	-	+	+	+	+

For growth on NA + 5% glucose, pale yellow and yellow colonies maintained their colour.

### 5.5. Citrate Utilisation Test

The result from Table 1 showed that Tafo 1 and 2, Ohawu 1, Sunyani, Nyankpala 1 and 2 and CRI 2 isolates showed positive result, this was an indication that they belong to *Xanthomonascampestris* group which was shown from the change of colour from green to blue. The Bonsu, Ohawu 2 and CRI 1 isolates showed negative result which means that there was no colour change from green to blue and they did not belong to the *Xanthomonascampestris* species. This was shown on Plate 5 below.

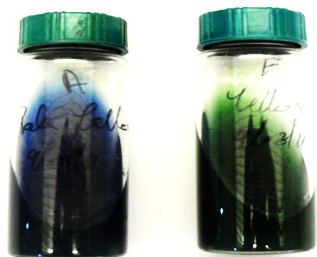


Plate 5. Citrate utilisation colour change

### 5.6. Salt Tolerance Test

From Table 1, all the pathogens grew in the salt solutions suggesting that they all belong to *Xanthomonascampestris* group. The growth of the pathogen in salt solution is shown in Plate 6 below.

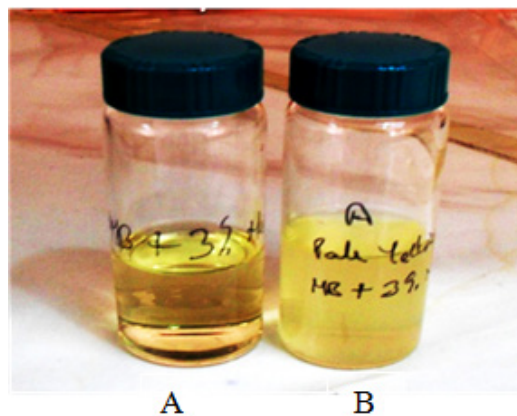


Plate 6. Bacteria blight isolate in salt solution; (A) control and (B) growth of inoculum

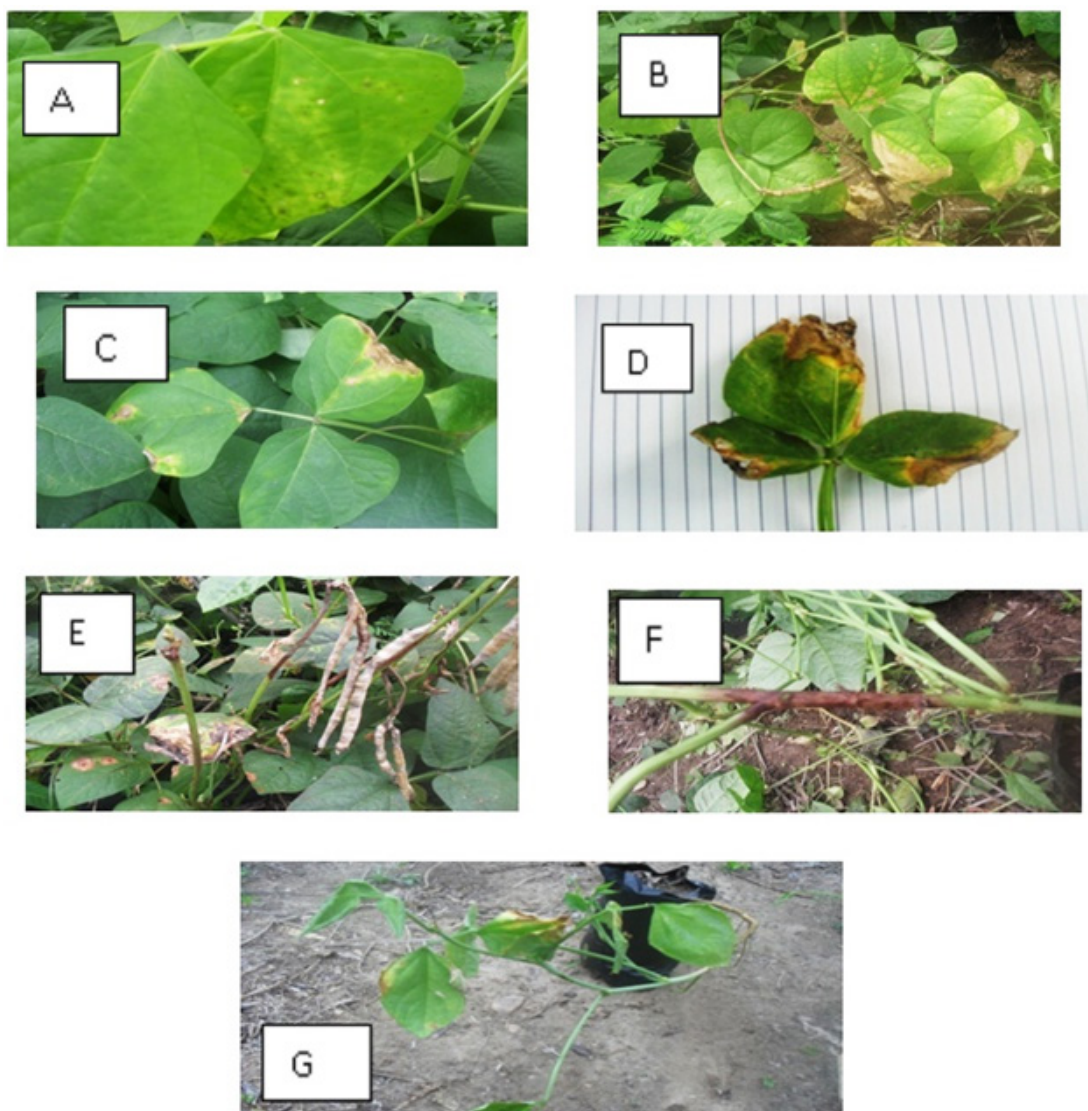


Plate 7. Levels of bacterial blight reaction symptoms: Water soaked spot, B) Development of yellow margin, C) Extension of the yellow margin. D) Brownish necrosis, E) Stem canker, F) Advanced stage of disease infection and G) Wilting

Cowpea bacterial blight (CoBB) symptoms start with small water-soaked spots on leaves which enlarge to irregular brown necrotic lesions surrounded by yellow haloes. The pathogen also invades cowpea stem causing canker symptoms on susceptible plants.

**Table 2. Disease severity and reaction of CoBB strain (Ohawu 1) to 31 cowpea genotypes**

Genotypes	Severity	Disease reaction
GH4025	1.0c	R
GH2347	1.3bc	R
IT97K-1069-6	1.3bc	R
Asontem	1.5bc	R
IT98K-131-2	1.5bc	R
GH2329	1.5bc	R
GH4778	1.5bc	R
GH4771	1.8abc	MR
IT97K-1113-7	1.8abc	MR
Local-2	1.8abc	MR
SARC1-57-2	1.8abc	MR
Ayiyi	1.8abc	MR
GH4229	1.8abc	MR
Marfo-Tuya	2.0abc	MR
Apagbaala	2.0abc	MR
GH3685	2.0abc	MR
UCC-White	2.0abc	MR
IT98K-506-1	2.0abc	MR
Nhyira	2.0abc	MR
SARC1-122	2.3abc	MR
GHTONA	2.3abc	MR
SARC-L03	2.5abc	MR
GH4765	2.5abc	MR
IT98K-499-35	2.5abc	MR
IT97K-568-18	2.8ab	MS
IT97K-1092-2	2.8ab	MS
UCC-Early	2.8ab	MS
GH7889	3.3a	S
IT84S-2246-4	3.3a	S
GH7225	3.3a	S
TVu7778	3.3a	S

Mean values in the same column followed by the same letter(s) are not significantly different at ( $P \leq 0.05$ ) using Duncan Multiple Rank Test: Severity, Disease reaction; R: resistant (severity score  $\leq 1$ ), moderately resistant ( $1.5 \geq$  severity  $\leq 1.8$ ), MS: moderately susceptible ( $1.8 \geq$  severity score  $< 3$ ); MR: S: susceptible (severity  $\geq 3$ ). Based on this classification, 64.5% showed moderate resistance, 22.6% were resistant and 12.9% were susceptible. The remaining strains of the bacterial blight isolate even though were pathogenic; there was no significant difference between the mean of the visual scorings at  $P \leq 0.05$ . The most pathogenic of all the strains was Ohawu 1 followed by Nyankpala 1 and CRI 2 respectively. Thermos resistant genotypes GH4025, GH4327 and IT97K-1069-6 exhibited higher level of resistant in all the three strains and the genotypes TVu7778, GH7889, IT84S-2246-4, and GH7225 exhibited similar levels of susceptibility to the three strains of the bacterial blight isolates.

Bacterial blight caused by *Xanthomonas axonopodis* sp. *Vignicola* (Xav) is one of the major diseases of cowpea giving rise to yield loss in all cowpea growing areas. The disease could be particularly devastating in drought-prone

areas of sub-Saharan Africa. The development of cowpea lines with resistance to this disease would be most attractive to farmers as a means of mitigating the adverse effects of the disease in cowpea.

Cultural methods such as intercropping cowpea with maize or cassava could also help to minimize yield losses due to the disease [28]. Previous research efforts on quick detection, identification, and characterization of Xav have been carried out [11,29], but the genetic inheritance of CoBB is still poorly understood. Bacterial blight symptoms were observed on leaves inoculated with  $10^8$  cfu  $g^{-1}$  bacterial suspensions. However, symptoms were observed on non-infected leaves of the susceptible lines IT84S-2246-4 and TVu7778 confirming that the disease is seed borne. Stem inoculation by inserting a sharp toothpick contaminated with bacteria blight suspension as suggested by [11,15] using two CoBB strains induced canker symptoms on stems in both susceptible and resistant cowpea lines tested. The absence of stem canker expression even in genotypes that showed high expression of leaf symptoms may indicate that different genes could be responsible for CoBB expression in leaf and stem. In an earlier study, [30] found cowpea varieties with leaves that were resistant to blight development while the stems showed canker expression. The author suggested that phytoalexins which confer resistance to the disease may be produced more in the leaves than in the stems of such varieties.

[27] reported that stem canker expression on cowpea is dependent on genotype. The results showed that only limited lesion areas were developed on resistant lines (GH4025, GH4327, and IT97K-1069-6) while lesion areas enlarged leading to leaf drop and in some cases death in most susceptible lines. This finding agrees with what is known about cowpea's defense response mechanism to *Xanthomonas axonopodis vignicola*, represented by a brown-red discoloration without a complete collapse of the tissue [9].

Plants employ a variety of defense mechanisms in response to pathogens, including the use of mechanical barriers, defense proteins and defensive enzymes [31]. A role for proteins in cell wall structure in bacterial blight disease resistance has been reported by different researchers in several plant species. In tomato, [32] suggested that the structure of pectin cell wall polysaccharides and specifically the degree of their esterification might play a role in defense against a bacterial pathogen. Plant peroxidases can be directly involved in defense mechanisms acting as catalysts for the polymerization of phenolic compounds to form lignin and suberin in the cell wall, which can act as barriers to block the spread of the pathogen in the plant [33]. However, [34] also suggested that peroxidases might play an important role in resistance to bacterial blight of cassava. A cationic peroxidase gene, MEPX1, was isolated from cassava and the DNA sequence of MEPX1 showed high homology with other plant peroxidase genes and contained a large intron typical of peroxidase genes [31]. The amino acid sequence had 75% homology with two *Arabidopsis thaliana* peroxidases. In cowpea, [35] detected a significant increase in  $H_2O_2$ -producing peroxidase (NADH-peroxidase) activity when cowpea plants were treated with  $H_2O_2$  at seedling and vegetative growth stages.



[35] observed that H<sub>2</sub>O<sub>2</sub> induces the synthesis of pathogenesis-related proteins, which help the plants to resist the pathogen attack. In cassava, [36] found that the production of phenolic compounds in the phloem and xylem of bacterial blight resistant cassava cultivars was significantly higher than in susceptible ones. There was also a higher accumulation of lignin and a greater formation of callose and tyloses in resistant cultivars which potentially obstruct the passage of the bacteria from cell to cell [36]. Also in cell walls of tomato genotypes resistant to *Ralstoniasolanacearum*, the cell wall structure was altered compared to susceptible genotypes, with differences in the distribution of acetyl esters of pectic polysaccharides [32].

## 6. Conclusion and recommendation

Among the cultivars tested the most pathogenic of all the strains was Ohawu 1, Nyankpala 1 and CRI 2 respectively. Results also show that the resistant genotypes GH4025, GH4327 and IT97K-1069-6 exhibited a various level of resistance in all the three strains and the genotypes TVu7778, GH7889, IT84S-2246-4 and GH7225 likewise exhibited various levels of susceptibility to the three strains of the bacterial blight isolated. It is recommended that a cowpea cultivar with resistance to both foliar and stem disease expressions is desirable.

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## Competing Interests

The authors declared that they have no competing interests.

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