Short communication

Evaluation of cowpea (*Vigna unguiculata* (L.) Walp.) landraces to bacterial blight caused by *Xanthomonas axonopodis* pv. *vignicola*

Hammed A. Durojaye, Yonnelle D. Moukoumbi, Victor O. Dania, Ousmane Boukar, Ranajit Bandyopadhyay, Alejandro Ortega-Beltran

**A R T I C L E   I N F O**

**Keywords:**
Disease resistance
Traditional landraces
Bacterial blight

**A B S T R A C T**

Cowpea is an important protein source for human populations in many nations across sub-Saharan Africa (SSA). However, cowpea production is constrained by bacterial blight (CoBB) caused by *Xanthomonas axonopodis* pv. *vignicola* (*Xav*), a disease affecting most cowpea-growing areas. A large proportion of smallholder farmers across SSA rely on traditional cowpea landraces (CLR) to produce the crop. The International Institute of Tropical Agriculture (IITA) possesses the largest collection of cowpea germplasm, including several CLR accessions. However, screening for resistance to CoBB in most of the CLR accessions maintained at IITA has not been conducted. CoBB severity was evaluated in 103 CLR accessions from five African countries, the US, the Philippines, and Sri Lanka by artificially inoculating a highly virulent *Xav* strain in plants grown in a screenhouse. Highly significant (*P* < 0.0001) differences in susceptibilities to the disease were detected among the evaluated germplasm. Resistance was detected in several CLR accessions with two accessions from Nigeria and one from the US developing no disease symptoms. Our results indicate that several CLR accessions are valuable sources of resistance to CoBB and those could be used to breed for improved varieties with superior resistance to the disease. The resistant CLR accessions and others in IITA collection should be further investigated to identify additional beneficial traits that may contribute to the development of improved, commercially acceptable varieties.

1. **Introduction**

Cowpea (*Vigna unguiculata* (L.) Walp.) is the most important legume native to Africa where is grown in the drier Savannas and Sahelian regions of sub-Saharan Africa (SSA). Those regions contribute circa 70% of global cowpea production (Boukar et al., 2012). Cowpea is also widely grown in certain nations of Latin America and Southeast Asia, and in the Southern US (FAO, 2016; Muchero et al., 2009). Across the world, over 12 million ha are cropped to cowpea with an annual grain production of > 6.9 million tons. The largest cowpea producers are Nigeria, Niger, and Brazil (FAO, 2016). Grains, leaves, and haulms of cowpea are valued for their nutritious content for humans and livestock. Grains are rich in protein—generally composed of 25% protein—and both macro and micronutrients; leaves and haulms also contain valuable nutrients and are used primarily as fodder for livestock (Singh, 2006). In SSA, cultivation of cowpea provides social and economic benefits (e.g. market access, registration of cooperatives, cash for social functions) to smallholder farmers due to its many uses (Kristjanson et al., 2005; Langyintuo et al., 2003; Langyintuo and Lowenberg-DeBoer, 2006).

Although cowpea is well adapted to most regions in SSA, the crop is threatened by several pests and diseases, including cowpea bacterial blight (CoBB), caused by *Xanthomonas axonopodis* pv. *vignicola* (*Xav*). The first report of the disease was done in the US during the mid-20th century (Nandini, 2012). In Africa, it was reported in 1964 in Tanzania (Allen, 1981) while in Nigeria it was first reported in 1975 (Williams, 1975). To date, CoBB has been reported in most nations where cowpea is grown (Rastas and Sahin, 2017; Moretti et al., 2007; Nandini and Kulkarni, 2016; Shi et al., 2016). The major impact of *Xav* infection is on the leaves and depending on the susceptibility of the genotype it can cause complete defoliation (Claudius-Cole et al., 2014). Pods, seeds, and stems are also affected.
Strategies to control CoBB include seed treatment with bactericides, intercropping, use of pathogen-free seeds, and use of resistant germplasm (Sikirou and Wydra, 2004). Use of chemicals may be too expensive for smallholder farmers and/or may not be readily available for them (Shi et al., 2016). Use of cowpea germplasm with resistance to CoBB is a promising strategy with the potential to control the disease in an economic and sustainable manner (Emechebe and Lagoke, 2002). Sources of resistance to various diseases are often found in landraces or wild relatives of crops (Hegde and Mishra, 2009). Across SSA, most cowpea growers rely on self-saved seeds of cowpea landrace (CLR) accessions that have been grown in traditional agro-ecosystems over hundreds of generations (Uguru, 1998). CLR accessions may harbour resistance to Xav, because of exposure to the pathogen over centuries and long-term selection of resistant accessions by farmers.

Seed is the primary inoculum source of Xav. Planting seeds infected with Xav can result in either pre- or post-emergence seedling infection and subsequent mortality (Ganiyu et al., 2017). CLR heirloom seeds are typically not tested for the presence of Xav or any other pathogen. Therefore, risks of CoBB outbreaks are prevalent. In addition, farmers using CLR seeds not infected with Xav may have their crops infected due to Xav infecting cowpea and/or alternative hosts in neighboring fields (Sikirou and Wydra, 2004).

IITA holds the world’s largest and most diverse cowpea collection, with over 15,000 unique accessions from 88 countries representing 70% of African cultivars and nearly half the global diversity (Boukar et al., 2012). Only a small fraction of the collection has been evaluated for resistance to CoBB. It would, therefore, be valuable to screen CLR accessions with the aim of identifying sources of resistance for either continuous usage as accessions with known resistance to CoBB or to be integrated into breeding programs for development of resistant, improved cowpea varieties. Based on these considerations, 103 CLR accessions were evaluated for resistance to CoBB under screenhouse conditions in the current study. The knowledge obtained from the current study will aid in detecting valuable CLR accessions possessing high levels of resistance to CoBB that could be integrated into breeding programs to develop cultivars with resistance to CoBB and other desirable traits.

2. Materials and methods

A total of 103 CLR accessions were evaluated for resistance to CoBB. The accessions, maintained at the IITA Gene Bank, were selected from a previous study examining the genetic diversity of CLR accessions for agromorphological descriptors conducted in IITA-Ibadan (M. Gedil et al., unpublished); the selected accessions are representative of the great diversity of the germplasm maintained at IITA. Accessions from Nigeria (52), South Africa (20), Tanzania (10), the US (9), Senegal (4), Uganda (4), The Philippines (2), Sri Lanka (1), and one of unknown origin, were used in the current study (Table 1). Two additional genotypes, Danila and IT84S-2246-4, which have been classified as resistant and susceptible to CoBB (Agbico et al., 2010), respectively, were provided by the IITA Cowpea Breeding Unit and were used as both positive and negative control treatments.

A Xav isolate obtained from a diseased cowpea plant grown in IITA experimental station at Minjibir, Kano State, Nigeria (12°10′42.0″N; 8°39′33.1″E), was used in the current study. The isolate, hereafter referred to as Xav-Minjibir, was highly pathogenic to diverse cowpea germplasm in studies conducted in our laboratory (unpublished). For inoculum preparation, Xav-Minjibir was grown on nutrient glucose medium (NG; 28 g l⁻¹ Nutrient Agar, 20 g l⁻¹ glucose) for 48 h at 28°C. Inoculum suspensions were prepared by harvesting bacterial cells into sterilized deionized distilled water. Suspensions were adjusted turbidimetrically using a spectrophotometer to an optical density of 0.050 nm (0.3) or, approximately 2.4 × 10⁸ colony forming units (CFU) ml⁻¹. CLR accessions were evaluated in their resistance to CoBB in a screenhouse at IITA-Ibadan (07°30′20.7″N; 03°54′08.4″E). Plastic pots

| Cowpea accession | Country of origin | Days to first disease symptom range | Disease severity 22 dai | Disease reaction |
|------------------|-------------------|-----------------------------------|------------------------|----------------|
| TVu 58, TVu 64   | Nigeria           | –                                 | 0.00 k                 | I              |
| TVu 102          | USA               | –                                 | 0.00 k                 | I              |
| TVu 10, TVu 42,  | Danila            | Nigeria                           | 4–22                   | 0.08 j-k       | R              |
| TVu 101          | Tanzania          | 4                                 | 0.08 j-k               | R              |
| TVu 97           | South Africa      | 12                                | 0.10 j-k               | R              |
| TVu 41, TVu 52   | Nigeria           | 4–22                              | 0.10 j-k               | R              |
| TVu 80, TVu 84,  | South Africa      | 4–12                              | 0.10 i-k               | R              |
| TVu 96           |                  |                                    |                       |                |
| TVu 4, TVu 11,   | Tanzania          | 4–22                              | 0.15 h-k               | R              |
| TVu 13, TVu 51,  | Senegal           | 4                                 | 0.16 h k               | R              |
| TVu 60, TVu 63   | Nigeria           | 4–7                               | 0.20 h k               | R              |
| TVu 63           | South Africa      | 4–12                              | 0.15 h k               | R              |
| TVu 76           | Senegal           | 4                                 | 0.20 h k               | R              |
| TVu 87           | Tanzania          | 4–22                              | 0.20 h k               | R              |
| TVu 71           | South Africa      | 4–7                               | 0.20 h k               | R              |
| TVu 81, TVu 92   | Nigeria           | 4–12                              | 0.25 h k               | R              |
| TVu 19, TVu 50   | Senegal           | 4                                 | 0.25 h k               | R              |
| TVu 69           | South Africa      | 4                                 | 0.27 h k               | R              |
| TVu 85           | Nigeria           | 4                                 | 0.30 h k               | R              |
| TVu 88           | Uganda            | 4                                 | 0.33 h k               | R              |
| TVu 2, TVu 49    | South Africa      | 4–12                              | 0.33 h k               | R              |
| TVu 32, TVu 90   | Tanzania          | 4–7                               | 0.33 h k               | R              |
| TVu 101          | Senegal           | 4                                 | 0.33 h k               | R              |
| TVu 98           | Sri Lanka         | 4                                 | 0.33 h k               | R              |
| TVu 76           | Nigeria           | 4–7                               | 0.38 h k               | R              |
| TVu 78, TVu 95   | Unknown           | 4                                 | 0.40 h k               | R              |
| TVu 35           | Tanzania          | 12                                | 0.42 h k               | R              |
| TVu 99           | Nigeria           | 4–12                              | 0.50 b-j               | R              |
| TVu 18, TVu 43,  | Danila            | 12                                | 0.50 b-j               | R              |
| TVu 47, TVu 67,  | Uganda            | 4                                 | 0.50 b-j               | R              |
| TVu 75           | 12                | 0.50 b-j                           | R                      |
| TVu 26           | Tanzania          | 12                                | 0.55 g-j               | R              |
| TVu 86, TVu 79   | Tanzania          | 4–7                               | 0.58 g i               | R              |
| TVu 8, TVu 9, TVu| Nigeria           | 4–12                              | 0.58 g i               | R              |
| 14, TVu 33, TVu  | Philippines       | 4                                 | 0.63 f i               | R              |
| 45               | Unknown           | 4                                 | 0.65 f h               | R              |
| TVu 104          | South Africa      | 4–7                               | 0.67 f h               | R              |
| TVu 77, TVu 82   | The Philippines   | 4                                 | 0.67 f h               | R              |
| TVu 22           | USA               | 4                                 | 0.67 f h               | R              |
| TVu 24           | Philippines       | 4                                 | 0.67 f h               | R              |
| TVu 57, TVu 62,  | Nigeria           | 4                                 | 0.67 f h               | R              |
| TVu 65, TVu 74   | Nigeria           | 4                                 | 0.67 f h               | R              |
| TVu 80, TVu 84,  | South Africa      | 4–12                              | 0.83 d h               | R              |
| TVu 10, TVu 13,  | Nigeria           | 4–12                              | 0.83 d h               | R              |
| TVu 20           | Nigeria           | 4–12                              | 0.86 c h               | R              |
| TVu 76           | Nigeria           | 4                                 | 0.91 c h               | R              |
| TVu 31           | Senegal           | 7                                 | 0.91 c h               | R              |
| TVu 25, TVu 29   | South Africa      | 12                                | 0.92 c h               | R              |
| TVu 36           | Tanzania          | 4–7                               | 1.00 b g               | MS             |
| TVu 100, TVu 103 | Nigeria           | 4–12                              | 1.00 b h               | MS             |
| TVu 16, TVu 17   | USA               | 4                                 | 1.00 b h               | MS             |
| TVu 27           | South Africa      | 4                                 | 1.00 b h               | MS             |
| TVu 40, TVu 94   | The Philippines   | 4                                 | 1.08 b-f               | MS             |
| TVu 21           | Philippines       | 4                                 | 1.08 b-f               | MS             |

(continued on next page)
of 20.3 cm in both diameter and height were disinfested with hot water, cleaned and filled with sun-dried, sterilized top loamy soil. Seeds were sown at 2.5 cm in depth on August 23 and October 24, 2016, for the first and second test, respectively. Three seeds per pot were planted and these were irrigated with tap water using a watering can every three days. The experiment was conducted twice over a two month period.

The experiments were arranged in a completely randomized design with three replications (one pot per replicate; each pot containing three plants) per accession. Six hours prior to the first inoculation and until completion of the evaluations, plants were misted with tap water using a watering can, in order to increase humidity in plant canopy.

Prior to inoculation, CoBB symptoms were not detected in any of the two tests, at 22 dai.

Disease severity values at the end of evaluations, 22 dai. Values are means of six replicates. Each replicate was composed of three plants. Means were separated using Tukey’s HSD test.

Accessions were classified as immune (I), resistant (R), moderately susceptible (MS), and susceptible (S) based on severity values. Water inoculated accessions served as negative control treatments. Accessions with the same severity values and originating from the same country are grouped in the same row.

| Cowpea accession | Country of origin | Days to first disease symptom range\(^a\) | Disease severity 22 dai\(^b\) | Disease reaction\(^c\) |
|------------------|-------------------|------------------------------------------|----------------------------|---------------------|
| TVu 72           | Senegal           | 4                                        | 1.08 b-f                   | MS                  |
| TVu 3            | Nigeria           | 7                                        | 1.13 b-f                   | MS                  |
| TVu 61           | Nigeria           | 7                                        | 1.16 b-e                   | MS                  |
| TVu 83           | South Africa      | 7                                        | 1.16 b-e                   | MS                  |
| TVu 1            | Nigeria           | 12                                       | 1.25 b-d                   | MS                  |
| TVu 28           | USA               | 4                                        | 1.25 b-d                   | MS                  |
| TVu 23           | USA               | 4                                        | 1.33 b-c                   | MS                  |
| IT84S-2246-4     | Nigeria           | 4                                        | 1.41 b                      | MS                  |
| TVu 30           | USA               | 7                                        | 2.00 a                     | S                   |
| TVu 46           | Nigeria           | 4                                        | 2.00 a                      | S                   |
| Danila water-inoculated | Nigeria | –                                        | 0.00 k                     | Resistant Control   |
| IT84S-2246-4 water-inoculated | Nigeria | 12                                       | 0.16 h-k                   | Susceptible Control |

\(a\) Days after inoculation (dai) in which symptoms appeared. ‘-’ indicates that plants of those accessions did not develop disease symptoms in any of the two tests, at 22 dai.

\(b\) Disease severity values at the end of evaluations, 22 dai. These are the averages of disease severity among the examined cowpea germplasm. At 22 dai, averages of disease severity values ranged from 0.0 (no detectable symptoms) to 2.0 (Table 1). Typical symptoms appeared in the form of small, water-soaked, brown lesions, which gradually expanded and coalesced to form large necrotic lesions. Symptoms were visible in some CLR accessions as early as 4 dai (Table 1). Based on disease severity values at the end of evaluations (22 dai), accessions were classified as immune, resistant, moderately susceptible, and susceptible to CoBB.

The susceptible group, with an average disease severity of 2.0, consisted of accessions TVu 30 and TVu 46, from the US and Nigeria, respectively (1.9% of evaluated germplasm; severity index = 2.0). The moderately susceptible group included 15 CLR accessions and the susceptible control IT84S-2246-4 (15.1% of evaluated germplasm; severity index range = 1.0–1.4). The resistant group was the largest and includes 83 CLR accessions and resistant control Danila (79.2% of evaluated germplasm; severity index range = 0.1–0.9). Remarkably, three CLR accessions, TVu 58 and TVu 64, from Nigeria, and TVu 102, from the US, exhibited no disease symptoms (2.9% of evaluated germplasm; severity index of 0.0, immune group). Water-inoculated Danila plants had no disease symptoms while water-inoculated IT84S-2246-6 plants had a disease severity index of 0.2 with symptoms appearing at 12 dai. The appearance of CoBB symptoms in water-inoculated IT84S-2246-6 suggests that seeds were contaminated with Xav, perhaps by an isolate with low virulence, although this was not tested.

Several resistant accessions had uniformly low disease severity values in both tests. Minor lesions occurred in accessions with disease severity indices of 0.1 and 0.2 (Table 1); TVu 41 and TVu 87 developed CoBB symptoms only at 22 dai. In general, for both susceptible and moderately susceptible accessions, CoBB severity progressed after 7 dai. CoBB reached its stationary phase at 19 dai in accessions within these categories and there were no significant (\(P > 0.05\)) differences in disease severity indices between the last two observation periods (data not shown).

4. Discussion

From the great diversity of cowpea germplasm maintained at IITA Gene Bank (Boukar et al., 2012), improved genotypes have been screened for resistance to CoBB and minor emphasis has been given to CLR accessions (Agbicodo et al., 2010; Sikirou et al., 2001). Knowledge of resistance of germplasm to important diseases is a valuable resource for plant breeding programmes because it helps to identify accessions in which to obtain genes associated with resistance. The current study evaluated variation in resistance to CoBB among 103 CLR accessions when challenged with a virulent strain of Xav. The evaluated accessions are maintained at IITA Gene Bank and constitute only a fraction of cowpea’s diversity. The large majority of the examined CLR accessions (79.8%) possessed resistance to CoBB. Three accessions (2.8%) did not express disease symptoms in any of the two tests. These CLR accessions are sources of resistance that should be considered for inclusion into breeding programs to develop a pipeline of inbred lines with high resistance to CoBB through conventional breeding and/or marker-assisted selection.

Although seed is the primary inoculum source of Xav (Ganiyu et al., 2017), our evaluations aimed to detect resistance to CoBB by directly...
inoculating leaves. Apart from the seed, Xav can overwinter on crop residues, fall-sown cereals, and perennial grasses and infection may occur after spread of bacterial ooze from diseased plants by raindrops, plant to plant contact, and insect transmission (Moretti et al., 2007; Sikiru and Wydra, 2004; Zandjanakou-Tachin et al., 2007). Inoculation methods used to screen for resistance to CoBB include inoculation, soil inoculation, stem injection, and foliar spraying (Kutama et al., 2013; Sikiru et al., 2001). The later was used in our study because it allows to inoculate leaves directly, without damage, and high disease pressure is provided.

Variability in disease symptom expression among the examined germplasm was detected (Table 1). Disease symptoms appeared as conspicuous yellow halos and chlorotic borders around necrotic lesions, which are typical of CoBB (Okechukwu et al., 2010). Some accessions exhibited symptoms at 4 dai while others had no symptoms even after 22 dai; CLR accessions (Tv58, Tv64, and Tv58) were completely resistant to CoBB. Leaf dropping occurred in some plants of most of the moderately susceptible and susceptible accessions. No trend was detected in which susceptibility categories were influenced by geographic origin of CLR accessions (Table 1). Our results identified several CLR accessions with superior genetic backgrounds that could lead to the identification of genes, quantitative trait loci (QTL), and/or single nucleotide polymorphisms (SNP) associated with resistance to CoBB. Future work evaluating resistance to CoBB in other sets of germplasm should consider including Tv58, Tv64, and Tv58 as resistant accessions and Tv46 and Tv30 as susceptible accessions.

CLR accessions Tv58, Tv64, and Tv58 should be integrated into breeding programs to develop cowpea improved germplasm. Indeed, the three accessions had greater resistance than resistant control Danila, a cultivar used in CoBB screening studies and development of markers associated with resistance to CoBB (Agbicodo et al., 2010). In the study conducted by Agbicodo et al. (2010) even the most resistant genotype, IT81D-1228-14, exhibited disease symptoms. However, in that study, two different Xav genotypes, Xav18 and Xav19, were used and it should be investigated whether the immunity observed in Tv58, Tv64, and Tv58 against Xav-Minjibir will hold against Xav18, Xav19, or other Xav genotypes. Uniformly low disease scores (i.e., with severity index of 0.1 and 0.2) were detected in 23 CLR accessions (Table 1). Those accessions should also receive consideration for integration into breeding programs, especially if possessing desirable agronomic traits. Future research efforts should investigate similarities in both resistance mechanisms and inheritance of resistance among the immune and resistant CLR accessions detected in the current study.

5. Conclusion

Millions of people across SSA and other regions rely on cowpea as a primary source of both food and income. Use of both immune and resistant CLR accessions identified in the current study should be promoted among cowpea growers to rapidly reduce incidences of CoBB. In addition, immune and resistant accessions should be integrated into traditional and/or molecular assisted breeding programs for the development of cultivars that can resist high pressures of Xav across SSA and elsewhere. Improved materials with resistance to CoBB would reduce losses associated with the disease. Landraces provide valuable sources of variation for bene-
Chitwood, J., 2016. Association analysis of cowpea bacterial blight resistance in USDA cowpea germplasm. Euphytica 208, 143–155.
Sikirou, R., Wydra, K., 2004. Persistence of Xanthomonas axonopodis pv. vignicola in weeds and crop debris and identification of Sphenostylis stenocarpa as a potential new host. Eur. J. Plant Pathol. 110, 939–947.
Sikirou, R., Wydra, K., Rudolph, K., 2001. Selection of cowpea genotypes resistant to bacterial blight caused by Xanthomonas campestris pv. vignicola. Plant Pathogenic Bacteria. Springer 309–314.
Singh, B., 2006. Recent progress in cowpea genetics and breeding, I international conference on indigenous vegetables and legumes. In: Prospectus for Fighting Poverty, Hunger and Malnutrition 752, Conference Publication Ed. Leuven, Belgium. International Society for Horticultural Science, Hyderabad, India, pp. 69–76.
Smykal, P., Coyne, C.J., Ambrose, M.J., Mxasted, N., Schaefer, H., Blair, M.W., Berger, J., Greene, S.L., Nelson, M.N., Besharat, N., 2015. Legume crops phylogeny and genetic diversity for science and breeding. Crit. Rev. Plant Sci. 34, 43–104.
Tanksley, S.D., McCouch, S.R., 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277, 1063–1066.
Uguru, M., 1998. Traditional conservation of vegetable cowpea in Nigeria. Genet. Resour. Crop Evol. 45, 135–138.
Wiesner-Hanks, T., Nelson, R., 2016. Multiple disease resistance in plants. Annu. Rev. Phytopathol. 54, 229–252.
Williams, R., 1975. Diseases of cowpea (Vigna unguiculata (L.) Walp.) in Nigeria. PANS Pest Artic. News Summ. 21, 253–257.
Zandjanakou-Tachin, M., Fanou, A., Le Gall, P., Wydra, K., 2007. Detection, survival and transmission of Xanthomonas axonopodis pv. manihotis and X. axonopodis pv. vignicola, causal agents of cassava and cowpea bacterial blight, respectively, in/by insect vectors. J. Phytopathol. 155, 159–169.