# Diversity Studies of *Xanthomonas citri* pv. *malvacearum* Strains Isolated from Cotton in Western Kenya Based on Rep PCR Analysis

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**Abstract**

*Cotton or Gossypium L. is an important fibre crop in the world. Prior to the 1990s, the cotton industry in Kenya used to be strong and it remains a very important cash crop to date. During the marketing year 1984/1985, annual yields as high as 70,000 bales of lint were reached. The 1990s was characterized with liberalization of the sector which resulted in near collapse of the sector. Production dropped significantly due to both biotic and abiotic factors. Cotton suffers from bacterial blight (BB), incited by Xanthomonas citri pv. malvacearum (Xcm) and is a key challenge facing the sector. It is an important disease with economic significance throughout all cotton growing areas in the world with lint yield losses ranging from 5- 35%. Cotton leaves clearly showing symptoms of BB infection were collected from the fields in Siaya and Busia of western Kenya. The leaves were first dried and necrotic parts were excised from the infected leaves and soaked in a bleach to kill the fungi. The treated lesions were cultured on nutrient agar (NA) at 26 °C. From the bacterial growth around the lesions, morphologically distinct colonies were taken and streaked on separate NA plates. Two freeze-dried Xcm cultures provided by BCCMTM/LMG were also used as a reference strains.40 Xcm bacterial isolates obtained were then analyzed by rep-PCR of BOX and ERIC (Enterobacterial Repetitive Intergenic Consensus)-PCR fingerprinting to determine the diversity of the Xcm bacteria collected from Siaya and Busia in Western Kenya based on principal component analysis (PCA). The analysis indicated there is sufficient diversity among the field isolates but there exists low molecular variance between Busia and Siaya populations of bacteria based on ERIC and BOX PCR analysis.*

**Key Words**: *Cotton; Bacterial Blight; Xanthomonas citri pv. malvacearum; rep PCR- BOX and ERIC; Kenya*

## INTRODUCTION

Cotton or *Gossypium* L. is the number one fibre crop in the world. In 2011, it was grown in the tropical and (sub) tropical regions of about eighty countries, China being the top producer with a share of approximately 27%. Prior to the 1990s, the cotton industry in Kenya used to be strong. During the marketing year 1984/1985, annual yields as high as 70,000 bales of lint were reached (Ikitoo, 2008; Ingram, 2005). With liberalization of the sector in 1991, governmental support was withdrawn, which largely contributed to the suspension of research activities and hence near collapse of the entire cotton industry (Cotton Development Authority [CODA], 2009). The cotton production dropped to a minimum as many farmers abandoned cotton and shifted to other crops. Although in 2006, the government started to take measures for reviving the sector, production still has to continue rising in order to meet the national demand and the country‘s true potential. In 2011, only 55,000 bales of lint per annum were produced, in contrast to a demand of 180,000 bales and a potential of 368,000 bales (CODA, 2009; Karuga, 2011; National Cotton Council of America, 2012; Omolo, 2006).

Taking into account this enormous scope for growth, the Kenyan government has classified cotton as a major sub-sector within ―Kenya Vision 2030‖ for addressing poverty (CODA, 2009). There are several arguments that indicate the sector‘s importance in poverty reduction, economic development and income increase, particularly in the rural areas. First of all, the cotton-to-textile/garment industry can help to increase employment since it has the potential to benefit one-quarter of the county‘s population, in a direct or indirect way. In addition, cotton is suitable for the marginal arid and semi-arid lands (ASAL), which cover approximately 87% of the country and don‘t allow other crops to perform well.

Meanwhile cotton suffers from many pests and diseases of which BB, incited by *Xanthomonas citri pv. malvacearum* (*Xcm*), is an important one with economic significance throughout all cotton growing areas in the world especially where climatic conditions are conducive, meaning hot and humid (Bayles & Verhalen, 2007; Delannoy et al., 2005). This bacterium can attack all aerial cotton parts in every phase of the plant‘s growth. The appearance of angular lesions between or concentrated along the leaf veins is called ―angular leaf spot‖ or ―vein blight‖ respectively. Black, stem girdling cankers found on the seedling hypocotyls and adult stems, branches and petioles is referred to as ―black arm‖. Infection of the fruits or bolls causes boll rot (Nyvall, 1999; Peeters et al., 2001; Sambamurty, 2006). Lint yield losses due to BB can range from 5 up to 30% or even more (Bayles & Verhalen, 2007).

For last 20-30 years, studies have gone beyond the phenotypic comparisons based on biochemical and physiological characteristics of bacteria (Parkinson et al., 2007; Van den Mooter& Swings, 1990; Vauterin et al., 2000). Analytical fingerprinting techniques such as protein electrophoresis (SDS-PAGE) and gas-chromatographic analysis of cellular fatty acid methyl esters (FAME) have been developed and applied for the taxonomic identification (Parkinson et al., 2007; Vauterin et al., 1991, 2000; Yang et al., 1993). A number of representative strains from those protein and fatty acid groupings were selected for a spectrophotometric DNA-DNA hybridization study (Vauterin et al., 1995, 2000), which resulted in the distinction of twenty constituent species by measuring levels of homology between complete genomes. This reclassification by Vauterin et al. (1995) tried to incorporate the existing pathovar system into a new genus structure. DNA-DNA hybridization is considered to be the standard for defining *Xanthomonas* species and assessing the relatedness between them (Parkinson et al., 2007; Vauterin et al., 2000). However, being very reliable, there are some limitations to the technique, including insufficient sensitivity to detect close relationships and laborious pairwise analyses which makes it unsuitable for routine identification (Parkinson et al., 2007; Vauterin et al., 2000).

Approaches based on the 16S rRNA gene or 16S-23S intergenic region of *Xanthomonas* could be more useful if it were not for the fact that the phylogenetic or taxonomic level of resolution is too low; with this method it is impossible to classify at the (sub) species level (Hauben et al., 1997; Parkinson et al., 2007; Rademaker et al., 2000; Vauterin et al., 2000).

Therefore, it was postulated and also confirmed by studies that genomic fingerprinting techniques such as AFLP and rep-PCR can be used as valuable, rapid and more facile alternatives to DNA-DNA hybridization (Rademaker et al., 2000, 2005). Rep-PCR is based on interspersed repetitive sequences which are highly conserved among several bacterial genomes. They are termed the repetitive extragenic palindromic (REP) sequence, the ERIC and the BOX element. Complementary primers amplify DNA fragments located between the repeated elements. This results in differently sized PCR products which can be separated by electrophoresis to give a good fingerprinting profile for diversity analysis (Louws et al., 1994; Versalovic et al., 1994; Zhai et al., 2010).

In Kenya, BB also seems to be a prevalent disease. However, little research has been done because of the low interest in cotton since its collapse in the 1990s. This is best exemplified with unavailability of data on *Xcm* in Kenya and the near denial by research centres and farmers of the presence of BB in the Kenyan cotton fields. The research stations are probably unaware of the disease incidence, but because more priority goes to important food crops such as wheat and maize, it maybe justifiable for misconception. The problems bedevilling many of the farmers, on the other hand, is ignorance of the disease, they observe the symptoms but mistakenly think they are related to natural leaf senescence and substantial yield loss may actually be due to this disease. This justifies the need to collect data on the presence, and diversity of *Xcm* in the cotton growing areas using modern fast PCR based methods the focus of this

study. These findings can be formulated into research and extension approaches that can assist to address the farmers‘ problems.

## MATERIALS AND METHODS

Cotton leaves clearly showing symptoms of infection by *Xcm* were collected from the fields in Siaya and Busia of western Kenya. The leaves were first dried and necrotic parts cut from the infected leaves and soaked in a bleach to kill the fungi. The treated lesions were placed on NA and maintained at 26 °C. From the bacterial growth around the lesions, morphologically distinct colonies were taken and streaked on separate NA plates. Two freeze-dried *Xcm* cultures provided by BCCMTM/LMG were also put on medium, with the intention of using them as a reference strains in the identification analysis.

### DNA Extraction

After growing the bacteria in nutrient broth at 26 °C for 24 hr, the entire suspension was vortexed and transferred to a 15 ml falcon tube. The bacterial cells were harvested by centrifuging then supernatant was discarded and the tubes were stored at -20 °C. When needed for DNA extraction, the bacterial pellet was brought at room temperature, thoroughly mixed with a pipette, transferred to a 1.5 ml tube and centrifuged. Subsequently, the total DNA was purified using the QiagenDNeasy 96 Blood & Tissue Kit (Venlo, The Netherlands). The dsDNA concentration was determined with dilution factor 40. High concentrations were adjusted to 100-200 ng/µl in order to meet the range requirements for the PCR. The purity of the DNA was also assessed, using the ratio of the absorbance at 260 and 280 nm. The purified DNA was kept at -20 °C for further analysis.

### Rep-PCR analysis of *Xanthomonas* isolates

Isolates that were detected as *Xanthomonas* were analyzed for their diversity by running a rep-PCR according to Zhai et al. (2010). The used primers were BOX (5‘-CTACGGCAAG-GCGACGCTGACG-3‘), ERIC1 (5‘-ATGTAAGCTCCTGGGGATTCAC3‘) and ERIC2 (5‘-AAGTAAGTGACTGGGGTGA-GCG- 3‘). They are complementary to repetitive sequences that are present throughout the bacterial genome. The total reaction volume was 25 µl, including 2 µl of DNA, 23 µl of a prepared reaction mixture of multiplex PCR master mix (containing Taq DNA polymerase and dNTPs), BOX and ERIC primer couples and milli-Q water. The thermocycler was set to run at the following conditions: Initial denaturation: 5 min at 94 °C, 35 cycles each consisting of: Denaturation: 30 s at 94 °C, Annealing: BOX: 1 min at 53 °C, ERIC1 & ERIC2: 1 min at 52 °C, Extension: 4 min at 72 °C and Final extension: 6 min at 72 °C.

After amplification of the DNA fragments located between the repetitive regions, these different sized products were separated by electrophoresis on a 2% agarose gel in TAE buffer. To enable a more precise estimation of the band sizes, a mixture of two Promega markers was used; 100 bp and λ DNA/hindIII. This combined ladder was loaded several times, for example every six samples. The gel had to run for 16 hr at a temperature of 4 °C and stained in ethidium bromide and finally visualized on a UV transilluminator.

The obtained rep-PCR banding profiles were used to determine the genetic diversity or relatedness among the different *Xanthomonas* isolates. These profiles were first converted to binary data of which a matrix of genetic distance could be generated in GenALEx Version 6.41. To visualize differences between the isolates and between the populations of Busia and Siaya, the strains were plotted in a two-dimensional principal coordinate graph by running a Principal Component Analysis (PCA) with the distance matrix as input (also in GenALEx). Last, an AMOVA analysis based on 999 permutations was performed to examine the degree of molecular variance among the two bacterial populations (Busia and Siaya). This variance, indicated by the PhiPT-value, was considered statistically significant when $P \leq 0.05$. The diversity analysis of the isolates was done on the combined BOX and ERIC data.

## RESULTS AND DISCUSSION

### Bacterial Culture and Isolation

After incubation of the sterilized lesions on growth medium, bacterial growth could be observed around the leaf parts (Fig.1). Most colonies seemed to be yellow or creamy in colour, sporadically an orange culture was seen. Although very unlikely to be *Xanthomonas*so were excluded, while isolates with distinct yellow or white colour were included in the further research. At the end of isolation and subculturing, 40 Kenyan isolates were retained for DNA extraction and storage on beads at -80 °C, supplemented with the two reference strains from the BCCM$^{TM}$/LMG collection.

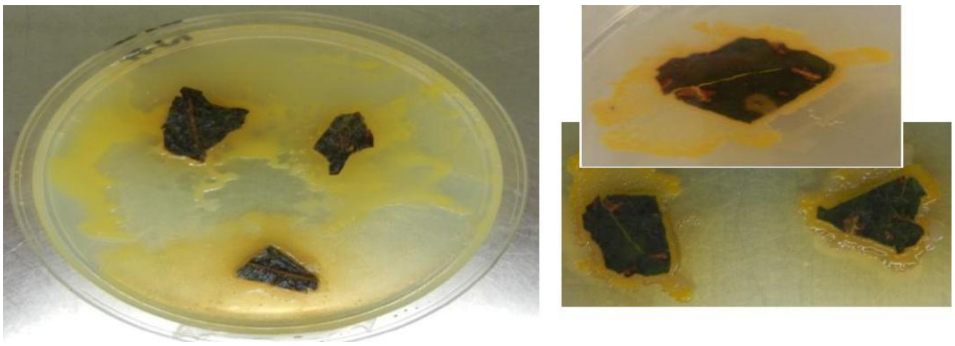

Figure 1. Bacterial growth around pieces of infected cotton leaves

### Rep-PCR analysis of *Xanthomonas* isolates

The BOX-PCR for the 40 identified *Xanthomonas* isolates yielded complex fingerprint patterns, with 29 different polymorphic markers that could be detected among all strains. The ERIC-PCR was performed to further evaluate the diversity since its primers target other repetitive sequences in the bacterial genome than the BOX-primers. A total of 28 polymorphic markers were detected by ERIC-PCR.

In Figure 2, the genomic fingerprints of the BOX-PCR are shown. The isolate number is indicated to facilitate the link with further statistical analysis. In between every six samples, a DNA ladder was inserted. The varying PCR profiles indicate there is sufficient diversity among the field isolates. Some pairs or groups of strains share many common bands or even complete patterns for example the strains 29 and 30 or 172 and 173.

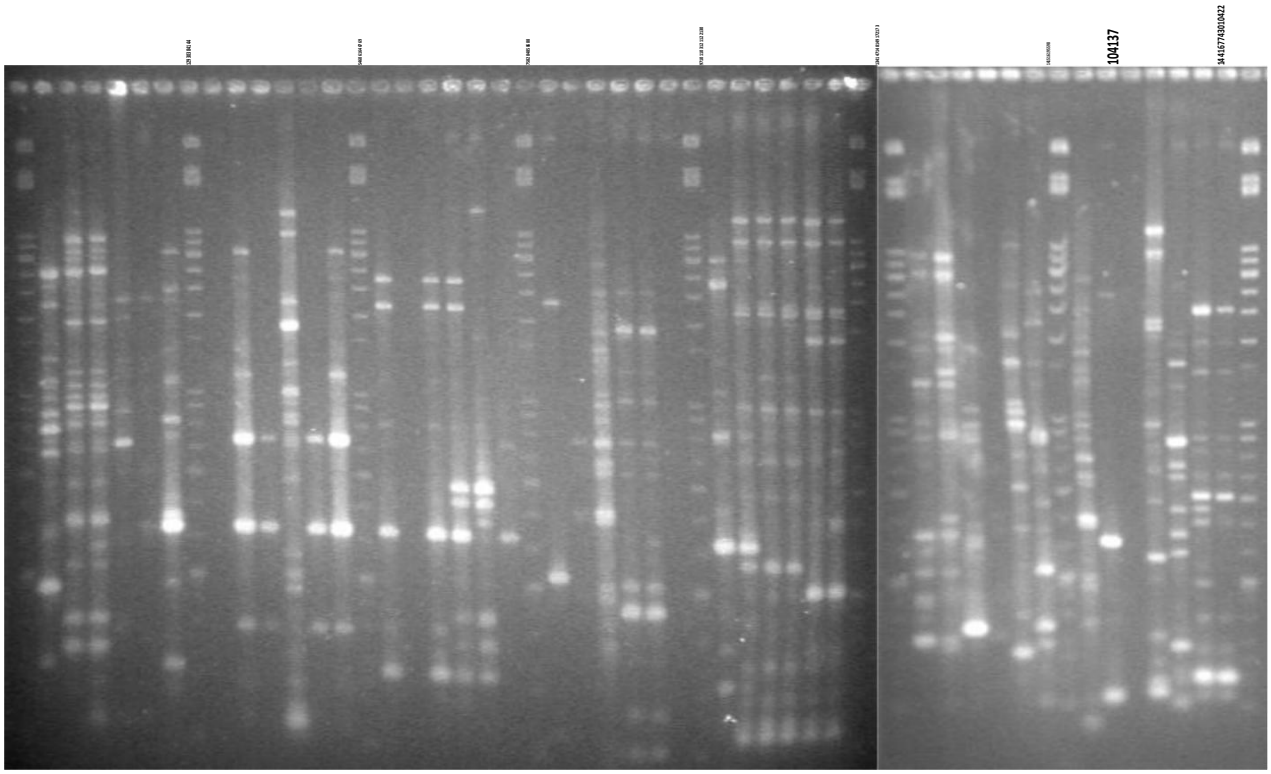


Figure 2. BOX-PCR fingerprint pattern of 40 identified *Xanthomonas* isolates (+ 2 LMG strains: 7430 &10422)

With the aid of the DNA ladder that was inserted several times, the position of the bands could be determined. Among all isolates, 29 and 28 different bands were identified with the BOX and ERIC-PCR respectively. This gave a total of 57 polymorphic markers for the combined analysis. By indicating per strain whether these bands were present or absent, a binary dataset was generated which was used for Principal Component Analysis (PCA). Isolates with an insufficient number of bands were left out. For diversity analysis, the results of both PCRs were combined, leading to the plot (Fig. 3). The X-axis explains 25.85% of the variation and the Y-axis 18.07%. A variance among the populations of 0.7% with a P-value of 0.318 proved that there is no difference between the Busia and Siaya bacterial populations. The low percentage of molecular variance between the two populations is probably due to the relatively short distance of approximately 60 km in between the two locations, the similar environmental conditions and the same cotton cultivar that is commercially grown in the entire area of Western Kenya (KSA 81M). It must be noted that the two counties of Siaya and Busia share a common border and also lie northeast of Lake Victoria with similar weather conditions (data not given) that may explain the low molecular variance.

The appearance of the dot plots in general reveals the fairly scattered position of the dots indicating the presence of molecular diversity within the collected *Xanthomonas* strains, which was already clear from the PCR banding profiles. No particular clustering is observed, with the exception of the isolates 147,148, 149, 172 and 173. Concerning the two LMG collection strains, they appear to show some degree of relatedness, being located at the margin of the point mass in the BOX and BOX+ERIC graph. However, all PhiPT- and associated P-values indicate there is a significant percentage of variance between the collection strains and the populations in Busia and Siaya.

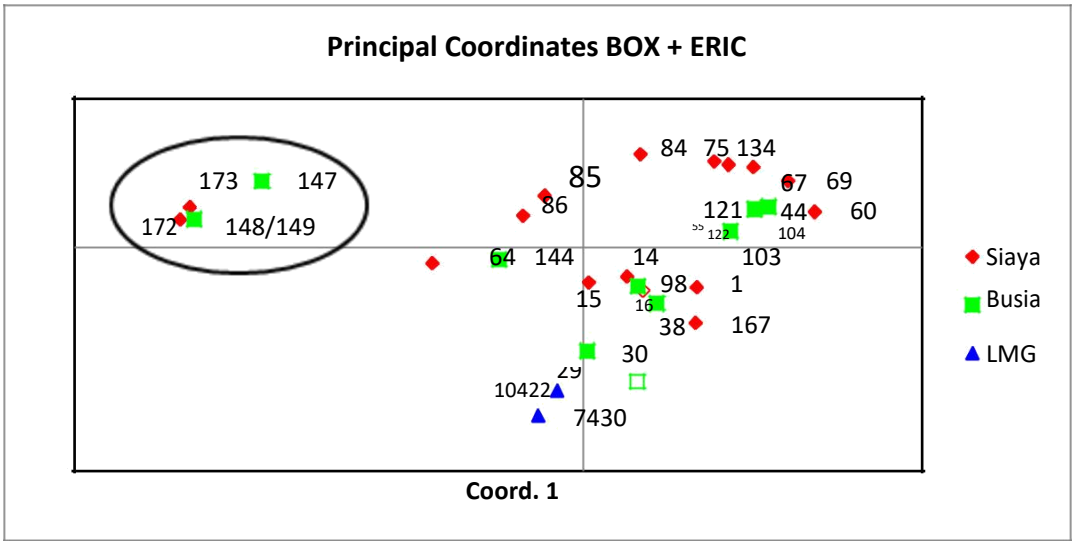


*Figure 3. Diversity analysis (PCA) of Xanthomonas isolates based on combination of BOX- and ERIC PCR; clustering of strains 147, 148, 149, 172 and 173 indicated with black circle*

Rep-PCR is a powerful tool for the molecular genetic analysis of bacterial taxonomy, and for diagnostic purposes in plant pathogenic bacteria especially at pathovar level and also for epidemiological analysis (Bruijn 1992, Louws et al., 1998). The rep-PCR technique has efficiently been used to fingerprint gram-negative bacteria, such as *Xanthomonas*, *Pseudomonas*, *Ralstonia*, and *Agrobacterium* (Bruijn 1992).

By using rep-PCR, strains of different *Xanthomonas* species were differentiated and the potential of rep-PCR in discriminating the strains has been proved in several publications. For instance, combined analysis of the PCR pattern obtained with REP, ERIC and BOX primers showed polymorphism among Brazilian *Xanthomonascampestris*pv*viticola* strains and allowed their separation into their respective subgroups (Trindade et al., 2005). Rep-PCR fingerprinting can be considered as an important tool for identifying and monitoring the diversity of the pathogen in a given affected region.

In our study, we explored the genetic diversity of Kenyan *Xcm* collected from cotton farms in western Kenya counties of Siaya and Busia and characterized using rep PCR (ERIC & BOX). Combined analysis of the PCR pattern obtained with ERIC and BOX revealed higher degree of genetic diversity among the ideotypes studied. The results further revealed lack of molecular variability between Busia and Siaya population of these isolates.

## CONCLUSION & RECOMMENDATIONS

The PCR profiles for *Xcm* field isolates obtained from western Kenya revealed varying DNA fingerprinting indicating there is quite sufficient diversity among the strains but the two populations from Busia and Siaya exhibit limited molecular variability. This may be attributed with the two counties shared common border, similar weather pattern and same variety of cotton grown across the region provided by one main supplier (CODA) probably justifying the low molecular variance among the two populations of bacteria based on rep-PCR of ERIC and BOX analysis.

This being the first molecular evaluation of *Xanthomonas* spp of cotton in Kenya will go a long way to understanding diversity of BB pathogen in the country but this would be useful if a corresponding status of resistance of cotton genotypes in Kenyan collection which will be done to help formulate breeding programs that are sustainable to addressing this problem. Identification of such resistance germplasm is very important in breeding for disease resistance. In addition a further differential race identification of *Xanthomonas* spp present in Kenya will be necessary since it will help to design specific breeding approaches targeting available races. Understanding the type of races found in this region will unravel an understanding of whether the strains present are unique for Kenya or particularly similar to other races found in other African countries. Therefore, as the Kenya government grapple with effort to revive the cotton industry, true recovery will be achieved with strong research backing addressing key challenges.

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## REFERENCES

Bayles, M.B. & Verhalen, L.M. (2007). Breeding and Genetics. Bacterial blight reactions of sixty-one upland cotton cultivars. *The Journal of Cotton Science*, *11*, pp. 40-51.

Bruijin, F.T. (1992). Use of repetitive (repetitive extragenic pallindromic and enterobacterial repetitive intergenic consensus) sequences and the polymerase chain reaction to fingerprint the genomes of *Rhizobium meliloti* isolates and other soil bacteria. *Applied Environmental Microbiology,* 58, 2180-2187.

CODA (2009). Developing cotton industry in Kenya [Pamba News Bulletin issue 001].

Delannoy, E., Lyon, B.R., Marmey, P., Jalloul, A., Daniel, J.F., Montillet, J.L., Essenberg, M. & Nicole, M. (2005). Resistance of cotton towards *Xanthomonas campestris pv. malvacearum. Annual Review of Phytopathology*, 43, 63–82.

Hauben, L., Vauterin, L., Swings, J. & Moore, E.R.B. (1997). Comparison of 16S ribosomal DNA sequences of all *Xanthomonas* species. *International Journal of Systematic Bacteriology*, 47, 328-335.

Ikitoo, E.C. (2008). Developing a sustainable production and marketing system for the cotton industry in Kenya. In ICAC, Improving sustainability of cotton production in Africa [Papers presented at the Technical Seminar at the 67th Plenary Meeting of the ICAC in Ouagadougou, Burkina Faso], pp. 9-17.

Ingram, M. (2005). Cotton-to-garment value chain analysis. In World Bank Group – Africa Private Sector Unit, Summary of Kenya Value Chain Analysis [Note Number 8], pp. 1-3.

Hauben, L., Vauterin, L., Swings, J. & Moore, E.R.B. (1997). Comparison of 16S ribosomal DNA sequences of all *Xanthomonas* species. *International Journal of Systematic Bacteriology*, *47*, 328-335.

Karuga, J. (2011). Renewed interest in cotton raises farmers' hopes. Available at: <http://www.businessdaily africa.com/Renewed+interest+in+cotton+raises+farmers+hopes/-/1248928/1091292/-/item/1/-/toiutm/-/index.html> [Accessed 10 August 2011].

Louws, F.J., Fulbright, D.W., Taylor Stephens, C. & de Bruijn, F.J. (1994). Specific genomic fingerprints of phytopathogenic *Xanthomonas* and *Pseudomonas* pathovars and strains generated with repetitive sequences and PCR. *Applied and Environmental Microbiology*, 60, 2286-2295.

Louws, F.J., Bell J., Medina-Mora C.M., Smart C.D., Opgenorth D., Ishimaru C.A., Hausbeck M.K., Bruijn F.J. & Fulbright D.W (1998). Rep-PCR-mediated genomic fingerprinting; a rapid and effective method to identify Clavibacter michiganensis. *Phytopathology*, 88, 862-868.

National Cotton Council of America. Cotton Crop Databases. Available at: <http://www.cotton.org/econ/cropinfo/cropdata/index.cfm> [Accessed 26 February 2012

Nyvall, R.F. (1999). Field Crop Diseases Third Edition. Ames: Iowa State University Press.

Omolo, J.O. (2006). The textiles and clothing industry in Kenya. In H. Jauch & R. Traub-Merz (Ed.), The future of the textile and clothing industry in Sub-Saharan Africa, pp. 147-164. Bonn: Friedrich-Ebert-Stiftung.

Parkinson, N., Aritua, V., Heeney, J., Cowie, C., Bew, J. & Stead, D. (2007). Phylogenetic analysis of *Xanthomonas* species by comparison of partial gyrase B gene sequences. *International Journal of Systematic and Evolutionary Microbiology*, 57, 2881–2887.

Peeters, M.-C., Van Langenhove, L., Louwagie, J., Waterkeyn, L. & Mergeai, G. (2001). 10. Fibre crops: Cotton – *Gossypium hirsutum* L.. In R.H. Raemaekers (Ed.), Crop Production in Tropical Africa, pp. 1041-1070.

Sambamurty, A.V.S.S. (2006). Bacterial diseases and plant galls. Chapter 9 in A Textbook of Plant Pathology, pp. 173-220. New Delhi: I.K. International Pvt. Ltd.

Rademaker, J.L.W., Hoste, B., Louws, F.J., Kersters, K., Swings, J., Vauterin, L., Vauterin, P. & de Bruijn F. J. (2000). Comparison of AFLP and rep-PCR genomic fingerprinting with DNA-DNA homology studies: *Xanthomonas* as a model system. *International Journal of Systematic Evolution and Microbiology*, 50, 665–677.

Rademaker J.L.W., Louws F.J., Schultz M.H., Rossbach U., Vauterin L., Swings J.& de Bruijn F.J. (2005). A comprehensive species to strain taxonomic framework for *Xanthomonas*. *Phytopatholology*, 95, 1098–1111.

Trindade, L.C, Lima, M.F, Ferrreira, M.A.S.V. (2005). Molecular characterization of Brazilian strains of *Xanthomonas campestris pv viticola* by rep –PCR fingerprinting. *Fitopatol Bras*, 30, 46 – 54.

Van den Mooter, M. & Swings, J. (1990). Numerical analysis of 295 phenotypic features of 266 *Xanthomonas* strains and an improved taxonomy of the genus. *International Journal of Systematic Bacteriology*, 40, 348-369.

Vauterin, L., Hoste, B., Kersters, K. & Swings, J. (1995). Reclassification of Xanthomonas. *International Journal of Systematic Bacteriology*, 45, 472-489.

Vauterin, L., Rademaker, J. & Swings, J. (2000). Synopsis of the taxonomy of the genus *Xanthomonas*. *Phytopathology,* 90, 677-682.

Vauterin, L., Swings, J. & Kersters, K. (1991). Grouping of *Xanthomonas campestris* pathovars by SDS-PAGE of proteins. *Journal of general microbiology*, 137, 1677-1687.

Versalovic, J., Schneider, M., de Bruijn, F.J. & Lupski, J.R. (1994). Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. *Methods in Molecular and Cellular Biology*, 5, 25-40.

Yang, P., Vauterin, L., Vancanneyt, M., Swings, J. & Kersters, K. (1993). Application of fatty acid methyl esters for the taxonomic analysis of the genus *Xanthomonas*. *Systematic and Applied Microbiology*, 16, 47-71.

Zhai, J., Luo, Y., Zheng, D. & Huang, X. (2010). Evaluation of Genetic Diversity of Highly Virulent Strains of *Xanthomonas campestris pv. malvacearum* by rep-PCR Fingerprinting. *Journal of Phytopathology*, 158, (11-12), 764–768.

## BIO-DATA

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