EVALUATION OF SEED DISSEMINATION STRATEGIES OF *XANTHOMONAS AXONOPODIS* PV. VIGNICOLA CAUSAL AGENT OF BACTERIAL BLIGHT OF COWPEA

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SUMMARY

Bacterial blight is seed transmitted but the mechanism of pathogen attachment to seed, their colonization of emerging seedling has not been fully studied. The aim of this study was to investigate the seed dissemination strategies of bacterial blight induced by *Xanthomonas axonopodis* pv. *vignicola*. Experiment to determine bacteria adhesion to seeds were conducted using twenty seeds each of Ife Brown (cowpea), Maize, Millet and Sorghum. The seeds were surface disinfected by washing in 3 % sodium hypo chlorite solution for 5 minutes and was rinsed SDW. Ten seeds each of the grains were put into a sterilized sample bottles containing 10 ml of bacterial suspension adjusted to *ca.*4.5x10^7 cfu/ml. Seeds soaked in SDW was used as control. The experiment to determine bacteria seed and seedling colonizing pattern were conducted using one hundred seeds of cowpea which were soaked in 100 ml of SDW containing *Xav* suspension adjusted to *ca.* 4.5 x 10^{7}cfu/ml^{-1}. Five seeds were placed in a Petri-dish containing SDW and spread filter papers. Seeds soaked in SDW were used as control. At different times after inoculation seeds were collected at random and ground, from which serial dilutions were made to quantify the population of bacterial colonizing the seeds/seedlings (cfu seed^{-1}). The result shows the adhesion of *Xav* to host's seed (cowpea) and non-host seeds (millet, sorghum and maize) after 4 h of inoculation. The result shows the

population of *Xav* was correspondingly increasing with the germinating seeds and growing seedling. This shows that distribution of bacterial blight is partly associated with the ability of the pathogens to adhere to seeds of both host cowpea and other seeds surfaces.

Keywords: Xav, Adhesion, colonization, nonspecific, cowpea seeds, transmission.

COWPEA (*Vigna unguiculata* L. Walp) is one of the most important legume crops grown in the tropical belt of Africa, Asia and South America (21). This crop provides food, animal feed and cash for the rural populace in addition to benefits to farmlands via *in situ* de-cay of root residues and ground cover due to the spreading habit of the plant. In addition, cowpea grain provides a cheap and nutritious food for relatively poor urban communities (17). The seeds are important in diets for the high protein content providing protein to rural as well as the urban dwellers as a substitute for the animal protein (24). Worldwide and in Africa, Nigeria ranked first in both production and consumption of cowpea (1, 8). Cowpea's high protein content, its adaptability to different types of soil and intercropping systems, its resistance to drought, and its ability to improve soil fertility and prevent erosion makes it an important economic crop in many developing countries. Increased and intense cultivation of any crop often results in an increase in pest pressure especially in the humid tropics. The major economic diseases of cowpea in the humid agro ecological regions of South-Western Nigeria include brown blotch, anthracnose, Cercospora leaf spot, web blight, sclerotium stem blight (1), cowpea aphid-borne mosaic virus, black-eye cowpea mosaic virus and cowpea mosaic virus (12), root-knot nematodes (16) and cowpea bacterial blight and bacterial pustule (6, 15).

The important bacterial diseases in Nigeria, are cowpea bacterial pustule (CBP) and cowpea bacterial blight (CoBB) are caused by different strains of *Xanthomonas axonopodis* pv *vignicola* (23). Bacterial blight occurs in all cowpea growing areas of Nigeria (7), causing severe grain yield loss of more than 64% in some areas of West Africa (19). When highly susceptible cultivars are sown, the crop may even be completely destroyed (7).

Disease incidence of cowpea bacterial blight is related to the seed borne nature of the pathogen with secondary spread occurring by winddriven rain (3). The bacterium can also survive in soil for up to eight months and in debris for longer periods (15). Symptoms of cowpea bacterial blight on leaves begin with small water-soaked spots, which remain small and, when the adjacent tissues die, gradually coalesce into large, irregular, brown, necrotic lesions surrounded by yellow haloes. The pathogen also invades the stem where it produces cracking with brown stripes, swelling (canker) and form dark green water-soaked patches on pods from where it enters the seeds and cause discolouration (3).

The management of the bacterial pathogen can be designed to reduce the multiplication rate at the site of infection on the host plant or to eliminate the inoculum at its source. Measures that affect pathogens at primary sources are more effective (9). It is necessary to ascertain the importance of the source in relation to the survival of the pathogen and the initiation of epidemics. A pathogen at its source is more effectively managed when detected early. Detection of the bacterium in seed and quick feedback on pathogenicity has a high impact on disease management options and cost of production. The movement of infected seed through international trade is also a major means of pathogen dispersal (20).

It has been established that CoBB is seed transmitted, and that seed-borne inoculum play a significant role in the disease epidemiology but the mechanism of pathogen attachment to seed has not been fully understood. The aim of the study was to investigate seed dissemination strategies of *Xanthomonas axonopodis* pv. *vignicola* causal agent of cowpea Bacterial Blight.

MATERIALS AND METHODS

Isolation of bacteria

Cowpea leaves showing symptoms of bacterial blight were collected from different fields. The leaves were placed in plastic bags and taken to the laboratory at the crop protection department faculty of agriculture Ahmadu Bello University Zaria. Surface sterilization with 0.5% NaOCl and rinsing in 3 changes of sterile distilled water was done thereafter 1- 2 mm leaf pieces were cut at the border of diseased portion. The pieces were ground in a porcelain mortar and pestle in 0.5 ml of sterile distilled water (SDW). Loop-fulls of the resulting suspension were streaked on to nutrient agar and incubated at 28 ^{o}C for 48-72 hours. Pure cultures were made from single colonies of bacteria. The isolates were stored on nutrient agar slants at 4^{0C} and later used for the inoculation of the seeds as well as control.

Bacteria adhesion to seed

Twenty seeds each of Ife Brown (Vigna unguiculata L.), Maize (*Zea mays* L.), Millet (*Setaria italica* L.) and Sorghum (*Sorghum bicola* L.), were surface disinfected by washing in 3 % sodium hypochlorite for 5 minutes and rinsed with SDW. Ten seeds each of the grains were put into a sterilized sample bottles containing 10 ml of bacterial suspension in SDW and concentration adjusted to *ca*.$4.5x10^7$ cfu/ml. Seeds soaked in SDW were used as control. These were left on the laboratory bench for 2 h and 4 h at room temperature (28^{0C}). After 2 h and 4 h the suspension was drained away and the seeds washed twice with SDW. Bacteria population sizes adherent on seeds were monitored by seed maceration and making serial dilutions and plating on nutrient agar to quantify the bacteria population. The percentage number of attached cells to the number of inoculated cells indicated capacities of the bacteria pathogen to the seed surface (4). Experiments were laid in complete Randomized Design (CRD) with five replications and repeated three time.

Colonization pattern of the pathogen on cowpea seedling

Seeds of Ife brown were surface disinfected before inoculation. One hundred seeds of surface sterilized cowpea were soaked in 100 ml of SDW containing *Xav* suspension adjusted to *ca*. 4.5 x 10^7cfu/ml^{-1}. After 4-5 h the seeds were removed from the suspension and dried at room temperature. Five seeds were placed in a Petri-dish containing moistened filter papers. Seeds soaked in SDW were used as control. At different times after placing seeds in the petri-dishes (24, 48 and 72 h) seeds were collected at random and ground, from which serial dilutions were made to quantify the population of bacterial colonizing the seeds (cfu seed^{-1}). The experiment was laid out with four replication and repeated three times. At 5, 7, and 9 days after inoculation, plants were withdrawn from the Petri dishes and were divided into: seed coat, cotyledons, hypocotyle, radicle, side roots, primar

y root and the emerging leaves, each were ground and serial dilution was made to quantify the bacterial population colonizing the various parts of the emerging plant (25).

RESULTS

Table 1 shows the adhesion of *Xav* to seeds. After 2 h of inoculation, cowpea had 100% adhesion, followed by millet (40 %), sorghum (20 %) and maize had no *Xav* adhesion. The *Xav* population was higher in cowpea, followed by millet and sorghum. At 4 h after inoculation all the seeds were attached by *Xav*. The highest adhesion was on cowpea (100%) followed by millet, sorghum and maize. There was however, statistical difference between *Xav* population on the seeds, ($P \leq 0.05$). Table 2 shows the result of colonization pattern of *Xav* on seedling. No *Xav* was detected on seed coat but at 5 DAI only was *Xav* detected on the cotyledons. The population of *Xav* was higher (10^7) on radicle, side roots and primary roots while that of hypocotyle has (10^6). The population of *Xav* was lower on the primary root at 5 DAI, similar result was observed of *Xav* population at 7 DAI. The population of *Xav* was lower on hypocotle (10^6) followed by emerging leaves (10^3). Figure 1 shows the result of progressive colonization of seeds/seedling over time. The population of *Xav* was correspondingly increasing with the germinating cowpea seedling.

Table 1: Adhesion of *Xav* to Seeds in 2011 Combine Analysis of three trials

Treatment	2 h		4 h	
	% adhesion	Population(cfu/seed)	% adhesion	Population(cfu/seed)
Maize	0.00d	0.00d	40d	$2.4x10^7$a
Cowpea	100a	$2.4x10^4$a	100a	$2.2x10^7$c
Millet	40b	$1.3x10^4$b	70b	$2.4x10^7$a
Sorghum	20.0c	$0.75x10^4$c	60c	$2.3x10^7$b
Control	0.00d	0.00d	0.00e	0.00d
S. E	0.65	0.05	0.84	0.07

Means in a column followed by the same letter are not significantly different at 5 % level of significance using Student-Newman-Keuls test.

Table 2: Colonization Pattern of *Xav* on Seed/Seedling (cfu/seed/seedling) in 2011Ccombine Analysis of three trials

DAI	Seed coat	Cotyledons	Radicle	Hypocotyle	Side roots	Primary root	Emerging leaves
5	-	$2.3x10^{2}$	$2.3x10^{7}$c	$1.4x10^{6}$c	$1.1x10^{7}$c	$2.6x10^{7}$c	$1.2x10^{3}$c
7	-	-	$2.5x10^{7}$b	$1.8x10^{6}$b	$1.4x10^{7}$b	$2.8x10^{7}$b	$1.3x10^{3}$b
9	-	-	$2.7x10^{7}$a	$1.9x10^{6}$a	$1.6x10^{7}$a	$3.2x10^{7}$a	$1.5x10^{3}$a
S. E			0.020	0.024	0.020	0.022	0.20

Means in a column followed by the same letter are not significantly different at 5 % level of significance using Student-Newman-Keuls test.

DAI = Days after inoculation; - = not detect

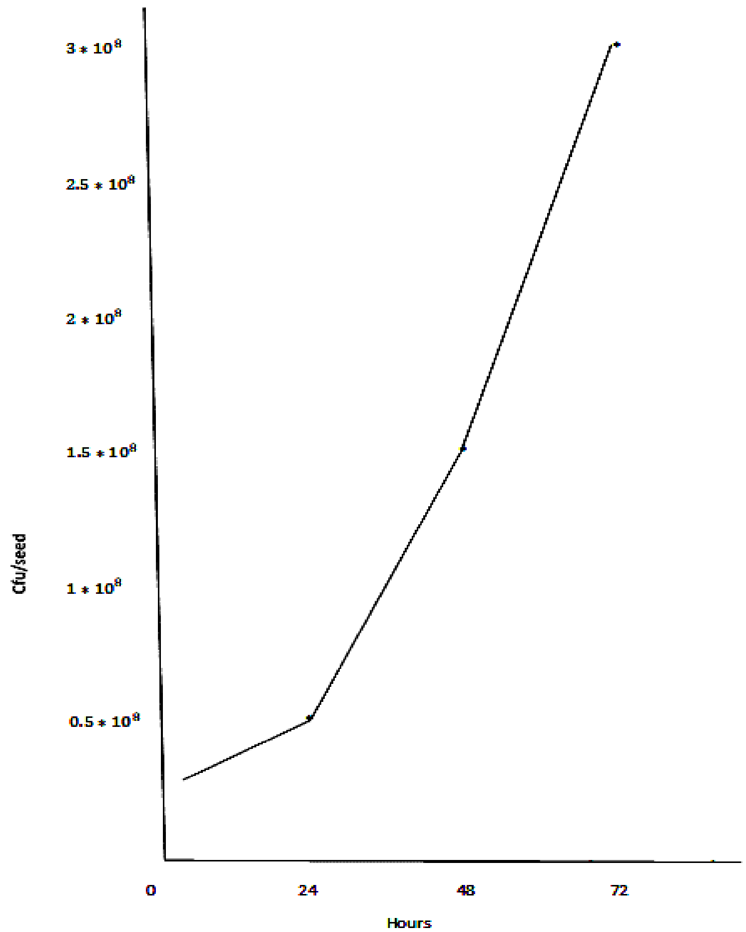

Fig 1: Progressive Increase of x av Population on cowpea Seed/Seedling Over Time

DISCUSION

Bacterial adhesion is the initial step in colonization and biofilm formation and it begins with the attachment of free-floating bacteria to a surface through weak, reversible van der Waal's forces (10). The 100 % of infection on cowpea seeds at 2 h after inoculation could be partly due to high imbibition of cowpea seeds that draw the bacteria to the seeds and tissue tropism (13, 22). Though bacteria attachment to surfaces appear to be nonspecific (4), particular bacteria pathogens are known to have an apparent preference for certain tissues over other. For example, *Salmonella mutan* is abundant in dental plaque but does not occur on epithelial surfaces of the tongue; the reverse is true for *Salmonella salivariu*s which is attached in high numbers to epithelial cells of the tongue but is absent in dental plague (22). The nonspecific adherence involves nonspecific attractive forces which allow approach of the bacterium to the eukaryotic cell surfaces. Possible interactions are (a) hydrophobic interactions (b) electrostatic attractions (c) Brownian movement and (d) recruitment and trapping by biofilm polymers interacting from fluctuating dipoles of similar frequencies (22). When the bacteria are not properly adhered or docked to the surface, it can easily slough, this explain why sorghum and maize seeds have little to no *Xav* attachment after 2 h inoculation (18, 22). The non-reversible (dock) attachment of bacteria cells to surfaces depend on surface properties of plant tissue, time, nutrient and water availability (18). The nature of the surface influence the attachment of the pathogen to the surface while rough and fleshy surface attract adhesion, the stony and leathery surface was not suitable for attachment (14). The stony and leathery surface were more pronounced on sorghum and maize seeds. The surface properties of seeds used in this work vary considerably. After 4 h of inoculation, all the seeds had *Xav* attached to it.

Individual bacterium coalesces by linking extra-cellar polysaccharides (EPS) on their cell walls. Polysaccharide chains exhibit chemical properties that make them polar and thus very “sticky”. This polarity is what leads to surface adhesions and cell cohesion and is one of the properties that make biofilms tough to remove (18). This result is in agreement with the report of Huber *et al*. (11) that many bacteria utilize sophisticated regulatory system to ensure that some functions are only expressed when a particular population density has been reached. The term “quorum sensing” has been coined to describe this form of density dependent gene. The little or no *Xav*. On seed coat and cotyledon of the germinating cowpea seedling might be of the available nutrients being used up by the germinating seedling making not much nutrient for the pathogen to feed on and multiply (2). Similarly, the corresponding increase in the population of bacteria cells with the germinating and growing cowpea seedling might be as a result of high nutrient exuding from the germinating and growing seedling (2, 25).

CONCLUSION

Essentially, the work has demonstrated that bacteria may nonspecifically attached on any surface exposed to some amount of water and nutrients. Once bacteria are attached to surface, they carry out a variety of detrimental or beneficial reactions depending on the species and on the surrounding environmental conditions. Bacterial adhesion to surfaces is the initial step in colonization and it begins with the attachment of free-floating bacteria to a surface through weak, reversible van der Waal's forces and sometimes strongly attached through biofilm formations. In addition, the study demonstrated ability of *Xav* to multiply as the seeds and seedling germinate and grow a strategy used by plant pathogenic bacteria to disseminate to the emerging plants. Bacteria need to be able to remain in, or in close contact to the seed, and to multiply there, before it can do harm to the germinating plant. The colonization of germinating seeds and seedlings represents a critical step in the establishment of bacterial disease; hence management strategies should aim at breaking the sequence of adhesion and colonization of the pathogen seeds and seedlings.

REFERENCES

1. **Adegbite A. A. and Amusa N.A. 2008.** The major economic field diseases of cowpea in the humid agro-ecologies of South-Western Nigeria. *African Journal of Biotechnology* 7. (25): 4706-4712.
2. **Bais H.P., Weis I. L. Pery L. G. Giluy S. and J. M. Vivanco 2006.** The role of root exudates in rhizosphere interactions with plants. *Annual Review of Plant Biology*, 57; 233-266.
3. **Claudius-Cole A. O., Ekpo E.J.A. and Schilder A. M. C. 2014.** Evaluation of Detection Methods for Cowpea Bacterial Blight caused by *Xanthomonas axonopodis* pv *vignicola* in Nigeria. Tropical Agricultural Research & Extension 17(2): 77-85.
4. **Darrasse A., Darsonval A., Boureau T., Brisett M., Durand K. and Jacques M.A. 2010**. Transmission of plant pathogenic bacteria by Non host Seeds without Induction of an Associated Defense Reaction at Emergence. *Applied and*

Environmental Microbiology 70 (20), 6787-6796.

5. **Darsonval A., Darrasse A., Durand K., Bureau C., Cesbron S. and Jacques M. A. 2009.** Adhesion and Fitness in the Bean Phyllosphere and Transmission to Seed of *Xanthomonas fuscans* subsp. *Fuscans. The American Phytopathological Society.* 22 (6) 747-757.
6. **Emechebe A.M. and Shoyinka S.A. 1985.** Fungal and bacterial diseases of cowpeas in Africa. Pp.173–197. In: Singh SR and KO Rachie (eds) Cowpea research, pro-duction and utilization. John Wiley & Sons Ltd., UK.
7. **Emechebe A.M. and Lagoke S.T.O. 2002.** Recent advances in research on cowpea diseases. pp 94-123.In: Fatokun CA, Tarawali SA, Singh BB, Kormawa PM, Tamo M (eds) Challenges and opportunities for enhancing sustainable cowpea production. Proceedings of the World Cowpea Conference III held at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, 4-8 September 200. IITA, Ibadan Nigeria.
8. **Food and Agriculture Organisation. 2011**. Accessed on June 2013. http://faostat.fao.org/site/339/default.aspx.
9. **Green, K. R. 1994.** Studies on the epidemiology and control of yam anthracnose. PhD thesis, University of Reading, UK. 190 pp.
10. **Hori K. and Matsumoto S. 2010.** Bacterial Adhesion: From Mechanism to Control.

Biochemical Engineering Journal,48,424-434. http://dx.doi.org/10.1016/j.bej.2009.11.014.

11. **Huber B., Eberl L., Feucht W. and Polster J. 2003.** Influence of polyphenols on bacterial biofilm formation and quorum-sensing. *Nature Force.* 58:879-884.
12. **Ittah M. A., Fawole I., Shoyinka S. A. and Hughes J. D. 2010**. Response of cowpea lines to inoculation with four seed trans-mitted viruses of cowpea. *Global Journal of Agricultural Science*. 9 (1): 27-33.
13. **Mah T. F. and O'Toole G. A. 2001.** Mechanisms of biofilm resistance to antimicrobial agents. *Trends microbiology*, 9: 34-39.
14. **Morais, L. C., Filho-Odilio R. B. and Assis B. G. 2009.** Wettability and bacteria attachmentevaluation of

multilayer proteases for biosensor application. *World Journal of Microbial Biotechnology*. 25; 123-129.

15. **Okechukwu R. U. and Ekpo E J. A. 2008.** Survival of *Xanthomonas campestris* pv. *vignicola* in infested soil, cowpea seed and cowpea debris. *Tropical Agricultural Research and Extension* 11: 43-48.
16. **Onifade A. K. and Fawole B. 1996**. Effect of some plant extracts on the pathogenicity of *Meloidogyne incognita* on cowpea. *Global Journal of Pure and Applied Sciences*. 2: 9-15.
17. **Quin F. M. 1997.** Importance of Cowpea p. 375-388. In: Singh, BBm Dashiell KE,. Mohan Raj DR and Jackai LEN (Eds.), Advances in Cowpea Research Colcor-craft, Hong Kong.
18. **Ramey B. E., Maria K., Bodman S. B. and Fuqua C. 2011.** Biofilm formation in plant microbe associations 2004: Retrieved May 17, 2011 from www.sciencediveit.com.
19. **Sikirou R. 1999.** Epidemiological investigations and development of integrated control methods of bacterial blight of cowpea caused by *Xanthomonas campestris* pv. *vignicola*. PhD thesis. University of Göttingen, Germany. p. 218.
20. **Singh S. P. and Muñoz C. G. 1999.** Resistance to Common Bacterial Blight among Phaseolus Species and Common Bean Improvement. *Crop Science* 39:80-89. doi:10.2135/cropsci1999.0011183X003900010013x.
21. **Singh B. B., Chambliss O. L. and Sharma B. 1997.** Recent advances in cowpea breeding pp 30–49. In: Singh BB, Mohan Raj DR, Dashiell KE, Jackai LEN (eds) Ad-vances in cowpea research. IITA and Japan International Research Centre for Agricultural Sciences (JIRCAS), Ibadan, Nigeria.
22. **Todar, K. 2011.** Mechanisms of bacterial pathogenecity. Retrieved June 14, 2011 from www.textbookofbacteriology.net .
23. **Vauterin L., Hoste B., Kersters K. and Swings J. 1995.** Reclassification of *Xanthomonas*. International Journal System Bacteriolology. 45:472–489.
24. **Wakili A. M. 2013.** Economic analysis of cow-pea production in Nigeria. *Russian Journal of Agricultural and Socio-Economic Sciences*, 1(13): 60 – 63.
25. **Yaryura P. M., Leon M., Correa O. S., Kerber N. L., Pucheu N.L. and Garcia A. F. 2008.** Assessment of the role of

chemotaxis and Biofilm formation as requirements for colonization of Roots and seeds of soyabean plants by *Bacillus amyloliguefaciens* BNM339. *Current Microbiology*.56:625-632