Methods for detecting the cassava bacterial blight pathogen: a practical approach for managing the disease

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Introduction

Cassava bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *manihotis (Xam),* is a destructive disease in South America and Africa that affects both yield and planting material leading to seed yield reductions. CBB is now a target for international phytosanitary quarantine efforts. The success of a cassava seed certification program depends on the availability of reliable tests to detect the pathogen.To prevent introducing the pathogen into disease-free regions, simple detection methods of *Xam* in plant tissues must be developed. These methods should be adaptable to the laboratories or institutional organizations concerned, depending on the facilities available.
Our objective is to report the recent developments of rapid and sensitive methods for detecting *Xam* in cassava tissues.

Fig 1. Cassava tissues used for detection studies (stems, fruits and seeds)

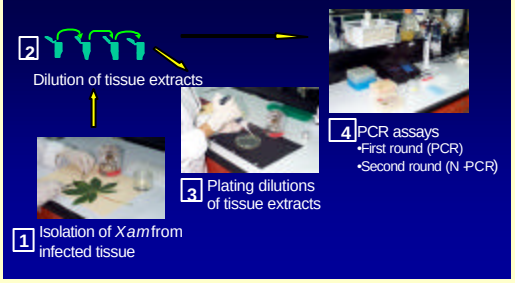
Materials and Methods

PCR assays. Various *Xam* bacterial strains, epiphytic bacteria isolated on cassava foliage as well as strains representing different pathovars of *X. axonopodis* were tested for primer specificity. PCR amplification assays are as previously described (Verdier et al 1998). To determine the detection level, dilutions of tissue extracts were plated on LPGA and the colonies that developed counted.

Nested-PCR assay. Nested-PCR is a PCR-based assay for which a second round of amplification is conducted by using primers internal to the first amplification product. Internal primers were designed. Twelve *Xam* strains and seventeen strains representing different pathovars of *Xanthomonas* were used. Non-inoculated and inoculated seeds and seeds collected from infected fields were used. PCR assays and nested-PCR assays are performed as previously described (Ojeda & Verdier 2000).

Dot-blot hybridization. Fruits and seeds are harvested and washed in sterile water; embryos are extracted from seeds and ground in sterile water. The washes of the fruits and seeds and the embryo macerates are serially diluted, to determine the presence of *Xam* colonies, and processed for dot-blot hybridization as previously described (Verdier & Mosquera 1999). A DNA fragment, 898-bp amplified by primers XV and XK is used for dot-blot assays.

FIG2. Scheme of the PCR and N-PCR detection assays.



Results

PCR assay.
Primer pair XV-XK directed the amplification of the 898-bp target sequence in DNA from *Xam* strains. No amplicons were produced from DNA extracted from other strains of bacteria. Extracts prepared from stem lesions were positive by PCR, with a bacterial concentration of about 1.2 x 10^3 CFU (colony forming unit) per reaction detected.

Nested-PCR.
The primers XVS2 and XKS2 directed the amplification of a 509-bp product in DNA from strains of *Xam.* Dilution series of freshly cultured cells of *Xam* strain OrstX 27 yielded a detection limit of 14 viable cells per reaction as determined by dilution plating on YPG agar. Ten seed lots were tested by PCR and nested-PCR. Results from seed washes indicated that 5.7% of the samples were positive for PCR and that 35.7% were positive for nested-PCR. The number of viable cells detected for nested-PCR ranged from 1.2 x 10^2 and 8.6 x 10^2 cells per reaction.

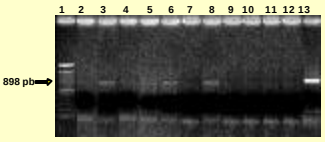


Fig 3. PCR amplification products from cassava seeds collected from naturally infected fields.

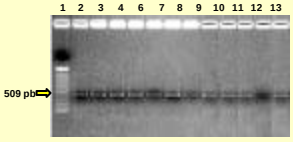


Fig 4. Nested-PCR amplification products from the PCR obtained in fig 3.

Dot-blot
Presence of *Xam* could be detected directly in cassava plant tissues (leaves, stems, fruits, seeds and embryos). The minimum number of cells detected was at 10^4 CFU/ ml, when 10 µl (10^2 CFU) were applied to the membrane. Of the fruit samples, 38% tested positive however the presence of large numbers of saprophytes made quantifying the pathogen difficult. Detection of external as well as internal contamination with *Xam* in seeds was successful, 45% and 36% , respectively, of tested samples were positive.

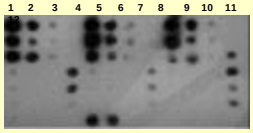


Fig 4. Dot-blot hybridization with the ^{32}P probe p898.

Table 1. Comparison of the different techniques for detecting *Xam.*

Technique	Costs		Labor	Detection
	Equipment	Consumables	Days required	sensibility
Culture medium	-	+++	4	++
PCR	++	++	1	+++
Nested-PCR	++	++	1	++++
Dot - Blot	+	+	2	++

Conclusions

- Different methods are available for detecting *X.a.* pv. *manihotis*. Specificity of these methods was tested with strains from South America, Africa and Asia, making these tests universal to use.
- The PCR procedure is simple to perform, and takes 2 h to complete. This rapid method is easily adapted for testing infected plant tissue and doesn't require prior DNA extraction.
- The nested-PCR increases sensitivity of detection and permitted to detect successfully the pathogen in seed washes, macerates, or embryos from seeds.
- The dot blot using the p898 fragment is a pathovar specific DNA probe. It is a simple and specific method for detecting *Xam* from plant tissues. It permits a large-scale testing of cuttings for culture indexing.
- These procedures can be easily implemented to certify seed lots, *in vitro* materials.

References

Ojeda S and Verdier V (2000). Can. Journal of Plant Pathology (in press).
Verdier V, Mosquera G and Assigbétsé K (1998). Plant Dis. 82: 79-83.
Verdier V and Mosquera G (1999). Journal of Phytopathology147: 417-423.